This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

COUNTWAY LIBRARY
HC 2AZN D

F. B. Mallory

THE BACTERIOLOGY OF DIPHTHERIA

CAMBRIDGE UNIVERSITY PRESS WAREHOUSE,
C. F. CLAY, MANAGER.
London: FETTER LANE, E.C.
Glasgow: 50, WELLINGTON STREET.

ALSO
London: H. K. LEWIS, 136, GOWER STREET, W.C.
Leipzig: F. A. BROCKHAUS.
New York: G. P. PUTNAM'S SONS.
Bombay and Calcutta: MACMILLAN AND CO., LTD.

[All Rights reserved.

THE BACTERIOLOGY OF DIPHTHERIA

INCLUDING SECTIONS ON
THE HISTORY, EPIDEMIOLOGY AND PATHOLOGY OF THE DISEASE,
THE MORTALITY CAUSED BY IT, THE TOXINS AND ANTITOXINS
AND THE SERUM DISEASE

By
F. LOEFFLER, M.D., LL.D.,
ARTHUR NEWSHOLME, M.D., F.R.C.P.
F. B. MALLORY, M.A., M.D.
G. S. GRAHAM-SMITH, M.A., M.D., D.P.H.
GEORGE DEAN, M.D.
WILLIAM H. PARK, M.D.
CHARLES F. BOLDUAN, M.D.

Edited by
G. H. F. NUTTALL, M.D., PH.D. SC.D., F.R.S.
QUICK PROFESSOR OF BIOLOGY IN THE UNIVERSITY OF CAMBRIDGE,
FELLOW OF CHRIST'S COLLEGE,
and
G. S. GRAHAM-SMITH, M.A., M.D.
UNIVERSITY LECTURER IN HYGIENE, CAMBRIDGE

CAMBRIDGE
at the University Press
1908

HARVARD MEDICAL LIBRARY
IN THE
FRANCIS A. COUNTWAY
LIBRARY OF MEDICINE

Cambridge:
PRINTED BY JOHN CLAY, M.A.
AT THE UNIVERSITY PRESS.

PREFACE.

WITHIN the last few years many admirable text-books of bacteriology have been published in the English language, which place before the student the essentials of bacteriological technique and the more widely accepted facts relating to the distribution and characters of the micro-organisms pathogenic to man and the domesticated animals.

In such works a single chapter is the utmost space which can be allotted to the consideration of a single organism, however great its importance, and much of this space is necessarily devoted to a description of its morphology and cultural characters. Questions dealing with the history of its discovery, mode of distribution, relation to other organisms and practical diagnosis, as well as those concerning the epidemiology, pathology, prevention and serum therapeutics of the disease which it produces, many of which are involved in great obscurity, have to be discussed in a few paragraphs.

Besides these works, which deal shortly with all the species of bacteria pathogenic to man, a few monographs and reports have been published dealing exhaustively with special points in the bacteriology or prevention of certain diseases.

To the advanced student, the bacteriologist, the physician, and medical officer of health these works are insufficient, and for further information he must turn to separate papers, scattered over a number of years throughout the various English and foreign journals and special publications, many of which are inaccessible. The desired references are frequently difficult to obtain and their verification involves the expenditure of much time and labour.

Much of the energy employed in these researches might be avoided if works dealing exhaustively with the bacteriological aspect of each infective disease were accessible.

The purpose of this volume is to partially fill this want by placing before the reader a full account of the bacteriology of diphtheria, the disease in which bacteriological methods are most commonly employed, not only in diagnosis but also in prevention and treatment.

The various chapters deal with the events which led up to the discovery of the diphtheria bacillus; the epidemiology and pathology of the disease; the morphological, cultural and pathogenic characteristics of the diphtheria bacillus and the organisms which resemble it, and their relationship to one another; the distribution of the diphtheria bacillus and organisms resembling it in men and animals; the occurrence of diphtheria and diseases simulating it in man and animals, and the relationship of the latter to diphtheria; the modes of infection and the methods of prevention; the practical and theoretical considerations involved in the manufacture of toxins and antitoxins and the results of antitoxin treatment.

Well-established facts have been described as briefly as possible, but where differences of opinion exist the views of both sides have been stated at length, and in all cases references are quoted for the statements which are made.

Though it is hoped that the reader will seldom find it necessary to refer to the original papers, a very full bibliography has been given at the end of the volume.

Except in regard to the characters of the membranes produced by the diphtheria bacillus and certain other organisms, the lesions due to injections of antitoxic sera (serum-sickness), and the occurrence of atypical forms of the disease, whose recognition is of importance in checking outbreaks, the clinical manifestations have not been discussed, as these are adequately dealt with in works on medicine.

The work is divided into six Sections, contributed by authors who have given special attention to the subjects discussed in each, and who are responsible for the opinions expressed.

We believe that the brief biographical notes accompanied by portraits of Bretonneau, who distinguished diphtheria as a specific affection and gave the disease its name, of Loeffler, who discovered the specific bacillus, of von Behring, who discovered the specific remedy, and of Roux, who practically applied the remedy to the cure of the disease in man, will be welcomed by many readers.

We desire to express our sincere thanks to Prof. Wesbrook for permission to reproduce plates VII and VIII (from *Trans. Amer. Pub. Health Assoc.* XXV. 1899), to Dr Denny for plate IX (from *Journ. of Med. Research*, IX. 1903), to Dr Hill for plate X (from *Annual Report of the Boston Board of Health*, 1901), to Prof. Abbott for plate XIII (from *Assoc. of Amer. Physicians*, 1893, and *Johns Hopkins Hosp. Bull.* IV. 1893), to Dr Gordon for figures 2 and 5, plate XII, and figures 1, 2 and 3, plate XIV (from *Local Government Board Report*, Appendix B, 1901–2), to Drs Weaver and Tunnicliffe for figures 5 and 6, plate XIV (from *Journ. of Infectious Diseases*, II. 1905), to the editors of the *Journal of Hygiene* for plates VI, XV and XVI, and to Dr James Ritchie for the photograph from which figure 1, plate XI, was reproduced.

In conclusion we desire to state that the University Press contemplate the publication of similar volumes dealing with the chief infective diseases, if this work meets with the approval of those for whom it is intended.

G. H. F. N.
G. S. G.-S.

CAMBRIDGE,
January 1908.

BRETONNEAU.

Pierre-Fidèle Bretonneau[1] was born 3rd April 1778 at St Georges-
r sur-Cher in the Province of Touraine. His father and several of his ancestors had been medical men. In 1795 he went to the École de Santé de Paris where he diligently studied chemistry, anatomy and surgery. After passing his medical examinations he went to Chenonceaux as "officier de Santé," but subsequently took his M.D. in Paris in 1815. He next became physician to the hospital at Tours, where he devoted his time to clinical and post-mortem work, paying special attention to the study of fevers. By the year 1818 he had become convinced that diphtheria in its various forms constituted a specific disease to which he gave the name "diphthérite." In 1825 he introduced the operation of tracheotomy. His writings exerted a great influence upon his contemporaries. He died at Passy 18th February 1862.

---

[1] A very full account of this eminent physician will be found in the following works, for the loan of which we are greatly indebted to Professor Roux.

Bretonneau et ses Correspondants, ouvrage comprenant la correspondance de Trousseau et de Velpeau avec Bretonneau publié avec une biographie et des notes par Paul Triare et précédé d'une introduction de L. Lereboullet, Vol. I. 596 pp. Paris. Félix Alcan.

Les Ancêtres de Bretonneau par le Dr Louis Dubreuil-Chambardel. 2ème. éd. avec Portraits. Paris. A. Maloine, 1905.

PIERRE-FIDÈLE BRETONNEAU,
born 3 April 1778,
died 18 February 1862.

FRIEDRICH AUGUST JOHANNES LOEFFLER.

LOEFFLER.

Friedrich August Johannes Loeffler was born 24th June 1852 at Frankfort a. O. He studied medicine in Würzburg and subsequently in Berlin. He served as a hospital assistant in the Franco-Prussian war. He graduated M.D. in 1874, and was subsequently stationed as a military surgeon in Hanover and Potsdam, where he practised medicine. In 1879 he was ordered to the Imperial Health Department together with Hüppe and Gaffky, and subsequently worked with Robert Koch until 1884. In 1886 he became Privatdocent in Hygiene in the University of Berlin, and two years later went to Greifswald as Professor of Hygiene. During the last few years he has also held the post of Rector of the University (1903–7). In 1905 he was raised to the rank of Generalarzt.

The importance of his bacteriological work, which has covered a very wide range, may be judged from the following brief summary of his discoveries. In 1882 he discovered the bacillus of glanders and shortly afterwards the bacillus of diphtheria. He also discovered the organisms which give rise to diphtheria-like diseases in pigeons and calves (1882), the bacilli which cause Schweinrothlauf (1885) and Schweineseuche, and the Bacillus typhi murium (1891), which has been extensively used in combating plagues of mice. He was the first to show experimentally that animals which have recovered from a bacterial disease are immune from a second infection with the same organism. In conjunction with Abel he demonstrated the formation of protective substances in the blood of animals treated with B. typhosus and B. coli. More recently (1897–1900) he has carried out extensive experiments on Foot and Mouth Disease. Besides the foregoing he has published numerous papers on disinfection, milk hygiene, sewage disposal, the bacteriology of water, a valuable work on the history of bacteriology (1887) and numerous reports and contributions on these and allied subjects to learned Societies, Congresses and Governments.

Bacteriological technique is greatly indebted to him for many of its standard methods.

In 1887 with Uhlworm and Leuckart he founded the *Centralblatt für Bacteriologie und Parasitenkunde.*

He has been the recipient of many honorary degrees from German and other Universities and many decorations from various Governments.

He is a Privy Councillor and a Member of the German Imperial Council of Health, etc.

BEHRING.

Emil von Behring was born 15th March 1854 at Hansdorf near Deutsch-Eylau in West Prussia. He studied at the Army Medical School in Berlin, graduating M.D. in 1878, and passing the "Staatsexamen" in 1880. He served in the army in Silesia and Posen, and was subsequently stationed at Bonn (1887), where he completed his first studies on rat serum in the Pharmacological Laboratory. In 1888 he was ordered to Berlin. In the following year he became assistant to Robert Koch at the Hygienic Institute, and subsequently worked under Koch at the Institute for Infectious Diseases. In 1893 the title of Professor was conferred upon him. During 1894 he temporarily filled the Chair of Hygiene in Halle, and in the following year became Professor of Hygiene in the University of Marburg, where he still occupies the position of Director of the Hygienic Institute.

Amongst his most important publications are papers relating to the action of iodoform and other disinfectants (1883—1890), and to the discovery of antitoxins for diphtheria and tetanus (in the latter case in conjunction with Kitasato), and the following books: *Die Blutserum-Therapie* (1892); *Gesammelte Abhandlungen zur aetiologischen Therapie von ansteckenden Krankheiten* (1893); *Die Geschichte der Diphtherie* (1893); *Die Bekämpfung der Diphtherie* (1894); *Allgemeine Therapie der Infektionskrankheiten* (1898); *Beiträge zur experimentellen Therapie* (1900–6); *Behringwerk-Mitteilungen* (1900–7). He has also published many shorter papers on experimental therapeutics in relation to anthrax and streptococcal infections, the hygiene of milk supply, water, etc.

A number of his more important addresses have also been published.

During 1901–4 he was chiefly occupied with experimental studies on the prevention of bovine tuberculosis. Since 1905 he has been engaged on studies on the collection of milk by aseptic methods, and its preservation by means of formalin and hydrogen peroxide. The results of some of these researches have already been applied in practice.

For his discovery of antitoxin he was awarded the prize of the Academie de Médecine and Institut de France (1895) and of the Senckenberg Institute of Frankfurt, etc.

He has been the recipient of many decorations and of many distinctions conferred on him by learned Societies.

In 1901 he received the patent of nobility, and in the same year was awarded the Nobel prize.

In 1903 he was raised to the rank of Wirklicher Geheimer Rath with the title of "Excellency."

EMIL VON BEHRING.

PIERRE PAUL EMILE ROUX.

ROUX.

Pierre Paul Emile Roux[1] was born 17th December 1853 at Confolens (Charente). He commenced to study medicine at Clermont-Ferrand, where he worked at chemistry under Duclaux (1872). At the conclusion of his medical studies in 1878 he entered Pasteur's laboratory at the École Normale and from that time till the death of Pasteur his scientific career was intimately associated with that of his renowned colleague. He collaborated with Pasteur in his researches upon the etiology of anthrax, the attenuation of various bacteria, and the use of attenuated cultures in immunisation (1876—1881). He assisted Pasteur in his studies upon rabies which led to the classical preventive treatment to that malady. His M.D. Thesis dealt with discoveries relating to rabies.

Since the foundation of the Pasteur Institute in 1888 he has devoted himself to technical microbiology, and the course which he started in that subject still continues. During 1887—1890 he published many papers either alone or in conjunction with Nocard, Chamberland and Yersin, the most important of which dealt with the asporogenic anthrax bacillus, the cultivation of the tubercle bacillus upon glycerinated agar, the immunisation against anthrax and the vibrio septique, and the diphtheria bacillus and its toxins.

In collaboration with L. Martin he prepared an antidiphtherial serum which gave such striking results in practice that doubt could no longer be entertained in regard to the value of the remedy. His report of the investigations conducted at the Children's Hospital in Paris was read before the International Congress of Hygiene and Demography at Budapest in 1894, where it created the greatest interest, and impressed all with its importance. The impression which it produced was in no small measure due to the lucid and convincing manner in which the report was presented by Roux. A fund was soon established by public subscription which enabled Roux to establish a special department for the preparation of curative sera.

Upon the death of Pasteur he became Subdirector of the Pasteur Institute, and took Pasteur's place in the Académie de Médecine. Since that time he has published important papers in collaboration with Metchnikoff and Salimbeni on cholera toxins and antitoxins (1896), with Borrel on the treatment of tetanus by intracerebral injections (1898) and with Nocard on pleuro-pneumonia in cattle (1899).

His latest researches conducted in collaboration with Metchnikoff deal with experimental syphilis.

In 1899 he was elected a member of the Académie de Sciences, and in 1904 he became Director of the Pasteur Institute upon the death of Duclaux.

---

[1] **We are much indebted to Dr Mesnil of the Pasteur Institute for the data upon which this biographical notice is based.**

G. H. F. N.
G. S. G.-S.

CONTENTS.

SECTION I.

By F. LOEFFLER, M.D., LL.D.

Privy Councillor and Professor of Hygiene in the University of Greifswald.

CHAPTER I.

THE HISTORY OF DIPHTHERIA.

The earliest records of diphtheria. The epidemic of the 16th century in Spain and the subsequent extension of the disease. Distribution of diphtheria in the 19th century. Earlier opinions on the nature of the disease. Experiments on animals. The rise of bacteriology. The discovery of the diphtheria bacillus by Loeffler. The investigations of other workers. Studies on diphtheria toxin. Local treatment of diphtheria by chemicals. The discovery of antitoxins and their application in the treatment of diphtheria by Behring, Roux and others . p. 1

SECTION II.

By ARTHUR NEWSHOLME, M.D., F.R.C.P.

Medical Officer of Health of Brighton.

CHAPTER II.

THE EPIDEMIOLOGY OF DIPHTHERIA.

Introduction. Diphtheria in the prae-statistical period. Diphtheria in the statistical period. Death-rate in England and Wales. Statistical experience of other countries. Determining causes of epidemics and pandemics. Deficient rainfall and diphtheria. Brückner's weather cycles. Influence of soil. Damp houses. Analogy with scarlet fever, rheumatic fever, etc. Urban and rural diphtheria. Geographical distribution of diphtheria. Relation of administration of antitoxic serum to epidemicity. Relation between prevalence and fatality. Natural variations in fatality. Seasonal incidence . . . . p. 53

SECTION III.

By F. B. MALLORY, M.A., M.D.

Associate Professor of Pathology, Harvard University Medical School; First Assistant Visiting Pathologist to the Boston City Hospital.

CHAPTER III.

THE PATHOLOGY OF DIPHTHERIA.

Introduction and literature. Technique; (*a*) Eosin and methylene blue stain, (*b*) Anilin blue stain, (*c*) Phosphotungstic acid haematoxylin stain. Varieties of lesions. A. *Local lesions*. Distribution of local lesions. Distribution of membrane. Gross characters of membrane. Nature of local lesions. Retrograde changes in the epithelium; (*a*) stratified squamous epithelium, (1) direct division, (2) vacuolation, (3) necrosis; (*b*) stratified cylindrical epithelium; (*c*) cubical epithelium. Exudation. The histological structure of membrane. The changes in the submucosa. The characters of membrane in different locations; (*a*) tonsils, (*b*) pharynx and soft palate, (*c*) epiglottis and larynx, (*d*) trachea, (*e*) tongue, (*f*) oesophagus, (*g*) stomach, (*h*) duodenum, (*i*) nose and accessory sinuses, (*j*) conjunctiva, (*k*) skin. Ulceration. The distribution of diphtheria bacilli; (*a*) in local lesions, (*b*) in the blood. The lungs; (1) bronchopneumonia, (2) atelectasis and emphysema, (3) interstitial tissue, (4) necrosis and abscess, (5) pleurisy, (6) bacteria. B. *Systemic or general lesions*. Introduction. The bone marrow; (*a*) nucleated red blood corpuscles, (*b*) bone marrow giant cells, (*c*) polynuclear neutrophile leucocytes, (*d*) endothelial cells, (*e*) eosinophile cells, (*f*) lymphoid cells, (*g*) plasma cells, (*h*) myelocytes, (*i*) premyelocytes and prelymphocytes. The blood. The lymphatic tissue; (*a*) lymph nodes, (*b*) tonsils, (*c*) thymus. The spleen; (1) lymph nodules, (2) pulp, (3) arteries. The gastro-intestinal tract. The heart; (1) fatty degeneration, (2) interstitial myocarditis, (3) mural thrombi. The kidneys; (1) degeneration changes, (2) acute interstitial changes, (3) acute glomerulonephritis. The liver; (1) degeneration of liver cells, (2) necroses of liver, (*a*) central necroses, (*b*) local necroses. The pancreas, salivary glands, adrenals, pituitary body, thyroid gland, and testicle. The nervous system. The skeletal muscles. C. *Secondary lesions*. (*a*) Accessory sinuses. (*b*) the middle ear, (*c*) acute abscesses, (*d*) general septicaemia, (*e*) acute endocarditis, (*f*) acute pericarditis, (*g*) acute pleurisy, (*h*) acute peritonitis, (*i*) acute suppurative mediastinitis, (*j*) other diseases. D. Repair, causes of death, sequelae, incubation. E. Experimental diphtheria . . . . p. 82

SECTION IV.

By G. S. GRAHAM-SMITH, M.A., M.D., D.P.H.

University Lecturer in Hygiene, Cambridge.

CHAPTER IV.

THE DIPHTHERIA BACILLUS.

Introduction. Morphology of the diphtheria bacillus in smears and culture. Size, shape. Staining of protoplasm. Classification. Involution forms. Characters at various periods of growth on serum. Post-fission movements. Branching forms. Spores. Reaction to staining agents. Loeffler's, Gram's, Nicolle's, Neisser's, Crouch's, Coles', Bronstein's, Cobbett's, Piorkowski's, Schauffter's, Falières', Ficker's De Rovaart's, Peck's, Pitfield's, Andrade's, Pugh's, Higley's, Ljubinsky's, Epstein's, and Boni's methods. Cultivation on artificial media. Loeffler's, Lorrain Smith's, Cobbett's, Kanthack and Stephens', Hayward's and White's serum media. Agar.

Gelatin. Potato. Broth. Products of growth on broth. Action on different sugars. Effects of alkalis and acids on growth. Milk. Litmus whey. Indol production. Vitality in culture. Chromogenic cultures. Influence of temperature on growth. Powers of resistance to cold, drying, light. Period of life in soil and dust. Effects of putrefaction. Action of germicides. Powers of resistance of bacilli enclosed in membrane. Behaviour in food substances: water, milk, butter, cheese, wine and bread. Virulence. Occurrence of diphtheria bacilli in notified persons, and "contacts"; (*a*) in families, (*b*) amongst attendants, (*c*) in hospital wards, (*d*) and schools. Distribution amongst healthy non-contacts. Summary . . p. 122

CHAPTER V.

THE PSEUDO-DIPHTHERIA OR HOFMANN'S BACILLUS.

Morphology. Reaction to staining agents. Involution forms. Appearances at different stages of growth. Post-fission movements. Branching forms. Spores. Influence of temperature. Cultivation on artificial media; serum, agar, gelatin, potato, broth, milk, litmus whey. Indol production. Virulence. Distribution. Seasonal prevalence . . . . p. 199

CHAPTER VI.

RELATION OF THE DIPHTHERIA BACILLUS TO HOFMANN'S BACILLUS.

Experimental attempts to convert the diphtheria bacillus into Hofmann's bacillus. Experimental attempts to convert Hofmann's bacillus into the diphtheria bacillus. Toxoid production by Hofmann's bacillus. Action of the bacilli on various sugars. Agglutination. Haemolysis. Experimental attempts to decrease the virulence of diphtheria bacilli. Experimental attempts to increase the virulence of diphtheria bacilli. Change of virulence by transference from one individual to another. Observations on the change of morphological type by various means. Hofmann's bacilli apparently pathogenic to man. The virulence towards guinea-pigs of Hofmann's bacilli derived from various sources. Experimental attempts to make Hofmann's bacilli virulent. Changes of morphological type in Hofmann's bacilli. Opinions of recent investigators. Summary . . . . . . p. 215

CHAPTER VII.

EXPERIMENTAL AND NATURAL DIPHTHERIA IN ANIMALS.

The lesions of experimental diphtheria in guinea-pigs, rabbits, dogs, rats, and mice. Experimental and natural diphtheria in cats, cows, and horses. Diphtheria bacilli in the blood and organs of experimental animals. Diphtheria-like bacilli found in animals. Experimental lesions in birds. Observations on the relationship of avian to human diphtheria. Holmes' observations. Summary . . p. 267

CHAPTER VIII.

THE MODES OF INFECTION IN MAN.

Transmission of the bacilli from patients, directly and through infected articles. Transmission of the bacilli by persons suffering from atypical forms of diphtheria; nasal catarrh, fibrinous rhinitis, tonsillitis, otorrhoea. Transmission of diphtheria by healthy contacts. Mode of transmission from unrecognised cases and healthy contacts. Infection through infected articles, food substances, milk, animals, birds, air, dust, soil, drains, sewer-gas. Laboratory infection. Summary . . p. 306

CHAPTER IX.

BACTERIOLOGICAL DIAGNOSIS.

Direct examination of cover-glass preparations. Swabs. Inoculation of culture media. Choice of media. Temperature and time of cultivation. Methods of making preparations for microscopical examination. Smears. Preparations from separate colonies. Staining of fixed preparations. Significance of polar bodies. The recognition of diphtheria bacilli under various conditions. Methods of obtaining pure cultures. The distinguishing cultural and pathogenic properties of the diphtheria bacillus. Methods of recording the morphological appearances. Unusual diphtheritic lesions of the mouth. Diphtheria-like organisms found in the mouth. Diphtheritic lesions of the nose. Diphtheria-like bacilli found in the nose. Diphtheria of the eye and diphtheria-like bacilli found in the eye. Diphtheria of the ear and diphtheria-like bacilli found in the ear. Diphtheria of the skin and diphtheria-like bacilli found on the skin. Diphtheria of the female genital organs and diphtheria-like bacilli found on them. Diphtheria of the male genital organs and diphtheria-like bacilli found in the urethra. Diphtheria of the anus and rectum. Diphtheria and diphtheria-like bacilli in the urine . . . . . . p. 330

CHAPTER X.

DIPHTHERIA-LIKE DISEASES.

Characters of diphtheritic lesions. Organisms which have been found associated with non-diphtheritic membranous lesions of the mouth, nose, and larynx; streptococcus, staphylococcus, Brisou coccus, pneumococcus, bacillus of Friedländer, bacillus coli, fusiform bacilli, spirilla, leptothrices and yeasts. Syphilis . . . p. 389

CHAPTER XI.

PREVENTIVE MEASURES.

General preventive measures. Notification. Isolation. Disinfection. Source of infection. Closure of schools. *Special preventive measures.* Primary and secondary examinations of clinical cases and contacts. Classes of persons who ought to be examined. Isolation of mild cases and infected contacts. Infected families. Schools. Isolation homes. Hospitals and institutions. Causes of misleading negative cultures. Proportion of negative, followed by positive, cultures. Persistence of diphtheria bacilli in the throat and nose. Attempts to hasten the disappearance of the bacilli. Results of isolation of convalescents, mild cases, and contacts in families, schools, institutions, hospital wards, and towns during outbreaks. The prophylactic injection of antitoxin. Summary . . . . p. 396

CHAPTER XII.

POST-SCARLATINAL DIPHTHERIA.

The prevalence of post-scarlatinal diphtheria. Origin. Treatment in the same hospitals of scarlet fever and diphtheria. Introduction of unrecognised cases. Results of bacteriological examinations. Spread of diphtheria in scarlet fever wards. Prevention . . . . p. 432

CHAPTER XIII.

DIPHTHEROID ORGANISMS IN THE INSANE.

The occurrence of diphtheriod organisms in general paralytics in the mouth, nose, genital and internal organs. Characters of the organisms. Eyre and Flashman's observations. Diphtheria bacilli in the tissues . . . . p. 441

SECTION V.

By G. DEAN, M.D.

Chief Bacteriologist, Lister Institute of Preventive Medicine, London.

CHAPTER XIV.

TYPES OF IMMUNITY.

Introduction and definition. "Virulence and toxicity." Natural immunity; (*a*) natural immunity in the individual, (*b*) racial immunity, (*c*) natural immunity in the species. Protective mechanism at the usual sites of diphtheria infection. Acquired immunity, active and passive. Inherited immunity . . . p. 449

CHAPTER XV.

THE TOXIN OF THE DIPHTHERIA BACILLUS.

The term "toxin." Discovery of diphtheria toxin. Toxicity of different races of diphtheria bacilli. Conditions which modify the virulence and pathogenic powers of the diphtheria bacillus; (*a*) association with other germs, (*b*) passage through animals. Influence of the medium on the production of toxin. Influence of temperature, oxygen, and light on the diphtheria bacillus. The period of maximum toxicity in cultures. Methods of testing the toxicity of the diphtheria bacillus. The relative susceptibility of different races of animals to diphtheria toxin. On the origin and nature of toxin. The constitution of the diphtheria toxin. Toxoids. The spectrum according to Ehrlich. Conditions which modify toxin; time, light, temperature, electricity, oxygen, peroxides and oxydases. Chemical agents. The purification and isolation of diphtheria toxin. The effects of toxin on animals. Subcutaneous injection. Latent period. General effect. Intracerebral injection. Physiological effects on the heart and circulation. The fate of the toxin *in corpore*; (1) in susceptible animals, (2) in refractory animals, (3) behaviour of toxin in the alimentary canal, (4) action of toxin on other mucous membranes . . p. 460

CHAPTER XVI.

THE FORMATION OF ANTITOXIN IN THE BODY.

Discovery of antitoxins. Active immunisation. Phenomena which occur during immunisation. "Marche ondulatoire." The technique of immunisation in animals (1) with toxin modified by (*a*) chemical agents, (*b*) heat, (*c*) antitoxin (toxone), (2) with toxin only. Discussion of the value of subcutaneous and intramuscular compared with intravenous injection of toxin. The period of maximum concentration of antitoxin in the blood. The operation of venesection. Variability in the capacity of horses to produce antitoxin. Subsequent history of diphtheria-antitoxin horses. Active immunisation in man. The effects of various chemical and physical agents on diphtheria antitoxin; heat, low temperature, light, gases, filtration. The testing of antitoxin. Ehrlich's method. The French methods. Attempts at the chemical isolation or concentration of antitoxin. The mechanism of antitoxin production. Ehrlich's theory . . . . p. 500

CHAPTER XVII.

THE EFFECTS OF ANTITOXIN ON TOXIN.

Theories regarding the interaction between antitoxin and toxin. Further analysis of the method of action of the antitoxin on the toxin. Brief summary of Ehrlich's theory of the relations of antitoxin to toxin. The physico-chemical theory of Arrhenius and Madsen. Criticisms of the views of Arrhenius and Madsen. The adsorption views of Bordet. The behaviour of antitoxin in the animal body, when used for the production of passive immunity. The disappearance of antitoxin from the blood. Absorption of antitoxin from the alimentary canal. The question of the production of anti-toxins. Inherited immunity. Transmission of inherited immunity. Antimicrobial diphtheria serum . . . . p. 538

SECTION VI.

By WILLIAM H. PARK, M.D.

Associate Professor of Bacteriology and Hygiene, University and Bellevue
Hospital Medical College, and Assistant Director of the Research
Bacteriological Laboratories of the Department of Health,
City of New York.

AND

CHARLES F. BOLDUAN, M.D.

Bacteriologist, Research Laboratory, Department of Health, City of New York.

CHAPTER XVIII.

MORTALITY.

The history of mortality from diphtheria before and after the introduction of the antitoxin treatment. The effects upon statistics of bacteriological examinations of suspected cases. The proportion of diphtheria cases sent to hospitals. The influence of the day of admission on the prognosis of hospital cases. The effect of heat and cold on the prevalence of diphtheria. The influence of altitude and soil on the prevalence of diphtheria. The influence of race, rainfall, and occupation. The mortality and prevalence of diphtheria at different ages. Infectivity and virulence. Characteristics of epidemics. Results of antitoxin treatment. Comparison of case mortalities. The influence of the time of administration on the value of antitoxin treatment. Regarding a possible decline in the virulence of the disease in modern times. Operative cases. Statistics on alternative cases. The absolute mortalities during a long series of years in large cities as an index of the value of antitoxin treatment. The extent to which antitoxin is used . . . . . . p. 574

CHAPTER XIX.

SERUM SICKNESS.

The clinical effects of diphtheria antitoxin. Short summary of the chronic toxic effects in man of injections of antitoxic or normal horse serum. The phenomena of serum sickness considered in detail. The stage of incubation. The fever. The rashes. The swelling of the lymph nodes. The blood changes in untreated diphtheria, in cases injected with antitoxic serum, and in cases developing serum sickness. Joint pains. Oedema and albuminuria. The condition of the mucous membranes. Rarer manifestations. Repeated injections of serum. Hypersensibility caused by serum injections. Late toxic effects produced in animals by the injection of heterologous serum. Differential diagnosis. The treatment of and prophylactic measures against serum sickness. The results obtained by the use of antitoxic globulin solution. Summary and conclusions upon the value of "refined" antitoxin. Calcium salts as a preventative of serum sickness. Theories as to the cause of serum sickness. Sudden death after an injection of serum. Duration of immunity. Repeated injections of antitoxin . . . . p. 614

BIBLIOGRAPHY . . . . p. 639

INDEX . . . . p. 705

LIST OF ILLUSTRATIONS.

Portrait of Pierre-Fidèle Bretonneau
" " Friedrich August Johannes Loeffler . . *between* pp. viii *and* ix
" " Emil von Behring
" " Pierre Paul Emile Roux . . . . . *between* pp. x *and* xi

Plate I. Fig. 1. Diphtheria Membrane. Direct division of nuclei of epithelial cells.
Fig. 2. Fibrinous membrane in the trachea . *to face* p. 88

Plate II. Hyaline membrane in pharynx . . . . *to face* p. 90

Plate III. Fig. 1. Hyaline membrane in stage of transformation from hyaline degenerated epithelial cells.
Fig. 2. Central necrosis of liver . . . . *to face* p. 92

Plate IV. Fig. 1. Focus of acute myocarditis.
Fig. 2. Acute myocarditis . . . . . *to face* p. 108

Plate V. Fig. 1. Peripheral nerve (popliteal) showing fat droplets in myelin sheath.
Fig. 2. Striated muscle showing fat droplets in fibres *to face* p. 112

Plate VI. Figs. 1—6. Forms of diphtheria bacilli . . *to face* p. 128

Plate VII. Figs. 1—9. A, B and C types of diphtheria bacilli according to Wesbrook, Wilson and McDaniel *to face* p. 130

Plate VIII. Figs. 1—10. D, E, F and G types of diphtheria bacilli according to Wesbrook, Wilson and McDaniel *to face* p. 130

Plate IX. Figs. 1—4. Diphtheria bacilli at various stages of growth.
Figs. 5, 6. Pseudo-diphtheria bacilli at various stages of growth.
Figs. 7, 8. Bacillus xerosis at various stages of growth *to face* p. 132

Plate X. Diagrams: Fig. 1. Illustrating "snapping" of diphtheria bacilli.
Fig. 2. Mode of division of B. typhosus.
Figs. 3, 4. Development of B. diphtheriae.
Fig. 5. Branching forms of B. diphtheriae *to face* p. 136

Plate XI. Fig. 1. Photograph of a smear preparation from the throat in a case of diphtheria.
Fig. 2. Photograph of groups of diphtheria bacilli in a section of a diphtheria membrane.
Fig. 3. Preparation of a pure culture of the diphtheria bacillus 24 hours old, stained by Neisser's method.
Fig. 4. Preparation from the same culture after 48 hours' growth *to face* p. 142

Plate XII. Fig. 1. Photograph of a pure culture of B. diphtheriae after 48 hours' growth on alkaline serum.
Fig. 2. Photographs of three serum cultures from the throat of a diphtheria patient.
Fig. 3. Photograph of colonies of B. diphtheriae on alkaline serum.
Fig. 4. Photograph of colony of B. diphtheriae on agar.
Fig. 5. Photograph of an agar culture of the nasal discharge of a case of acute coryza, showing colonies of B. Hofmanni and B. coryzae segmentosus . . *to face* p. 148

Plate XIII. Fig. 1. Area of acute necrosis at the seat of inoculation in a cow. B. diphtheriae seen in clumps.
Fig. 2. Section of focus of leucocytes in the omentum of a guinea-pig.
Fig. 3. Hyaline muscle at the seat of inoculation showing penetration of diphtheria bacilli (guinea-pig).
Fig. 4. Contents of one of the foci shown in Fig. 3 dried on a cover-glass . . . . *to face* p. 272

Plate XIV. Fig. 1. Photograph of a smear preparation of B. coryzae segmentosus.
Fig. 2. Chromogenic diphtheria-like bacillus from the eye.
Fig. 3. Diphtheria-like Streptothrix from the throat.
Figs. 4—6. Bacillus fusiformis . . . . *to face* p. 348

Plate XV. Fig. 1. Hofmann's bacillus (pseudo-diphtheria type).
Fig. 2. Hofmann's bacillus (common type).
Fig. 3. B. diphtheroides citreus.
Fig. 4. B. diphtheroides brevis.
Fig. 5. B. maculatus.
Fig. 6. B. diphtheroides liquefaciens . . . *to face* p. 352

Plate XVI. Fig. 1. B. xerosis.
Fig. 2. B. xerosis canis.
Fig. 3. B. auris.
Fig. 4. B. ceruminis.
Fig. 5. B. diphtheroides gallinarum.
Fig. 6. Diphtheria-like bacillus from the urethra *to face* p. 362

SECTION I.

CHAPTER I.

THE HISTORY OF DIPHTHERIA.

By F. LOEFFLER.

The earliest records of diphtheria, p. 1.—The epidemic of the 16th century in Spain and the subsequent extension of the disease, p. 6.—Distribution of diphtheria in the 19th century, p. 15.—Earlier opinions on the nature of the disease, p. 16.—Experiments on animals, p. 18.—The rise of bacteriology, p. 21.—The discovery of the Diphtheria bacillus by Loeffler, p. 28.—The investigations of other workers, p. 37.—Studies on diphtheria toxin, p. 45.—Local treatment of diphtheria by chemicals, p. 47.—The discovery of antitoxins and their application in the treatment of diphtheria by Behring, Roux and others, p. 48.

The Earliest Records of Diphtheria.

How long has diphtheria been known as a specific disease? Were the old physicians of the school of Hippocrates acquainted with it? The difficulty of answering these questions can be appreciated when we remember that in modern times the clinical features of diphtheria were first clearly grasped and described by Bretonneau in the early part of the last century, and that more than fifty years elapsed before the discovery of the causative organism enabled us to differentiate clearly this disease from those which resemble it. On the one hand the occurrence of epidemics, especially amongst children, and the characteristic clinical manifestations, render diphtheria an easily recognisable disease even to laymen, and we are consequently forced to the conclusion that the old Greeks, Hippocrates and his pupils, had no knowledge of it. We find in the writings of these physicians the most exact and minute accounts of various diseases of the throat, pharynx, and air-passages, but not one of these accords with the picture of diphtheria, and if it had occurred such excellent observers would not have failed to recognise it. Even in the writings of Celsus, Soranus, Galen and Caelius Aurelianus no mention is made of this disease.

On the other hand the disease appears to have been perfectly well known in Egypt, Syria and Palestine, even in ancient times. This is proved by the repeated references to it in the Babylonian Talmud (Wünsche 1886, Strack 1894)[1], a work, which, though it only appeared in the fifth century, transmitted the old Jewish traditions. In the Berachoth Treatise the following passage occurs: "There were created 903 kinds of death in the world...the hardest of all angina (according to Strack "Askara"), the easiest of all Kuss. Angina is like a brier in a bundle of wool which one casts behind one (for it is as difficult for the soul to leave the body as it is to remove a brier from a bundle of sheep's wool). According to some it is like unto a ship's cable in the opening of the throat (for a ship's cable can only be drawn with difficulty through a small hole). The Kuss is like unto a hair which one draws out of milk."

Askara we should now translate as diphtheria. According to Strack the word can be connected with the verb *śākhár*, to stop up, and would therefore designate a disease which chokes.

This view is supported by other passages in the Schabbath[2] and Thaanith[3] Treatises.

---

[1] See Bibliography.

[2] "Die Rabbinen haben überliefert: Die Bräune kommt in die Welt nur wegen des Zehnten. Nach R. Eliaser bar R. Josse kommt sie wegen der Verleumdung. Raba oder, wie manche sagen, R. Joshua Ben Levy hat gesagt: 'Aus welcher Schriftstelle lässt sich das beweissen?' Aus Psalm 63. 12: 'Und der König freut sich in Gott, es rühmt sich, wer ihm Treue schwört, denn er vertilgt den Mund der Verleumder,' oder nach Strack: 'denn verstopft werden soll der Mund der Lügenredner.'"

That the disease was one which specially menaced children is clear from the following passage (fol. 33*a*—34*a*):

"Hierauf nahm R. Simeon das Wort und sprach: 'Sie kommt wegen der Sünde der Vernachlässigung der Thora.' Da hielten sie ihm ein: 'Frauen, welche ihre Männer, Fremde, welche Israel, und Kinder, welche ihre Väter vom Thorastudium abhalten, beweisen doch das Gegenteil? Ferner beweisen doch auch Schulkinder das Gegenteil? Nein, es ist so, wie R. Gurjon erklärt hat; denn dieser oder, wie andere meinen, Rab Joseph bar Schemaja hat gesagt: 'Zu der Zeit, wo Gerechte im Zeitalter sind, da werden diese für das Zeitalter in Anspruch genommen (sie leiden für das Geschlecht); sind keine Gerechten im Zeitalter da, so werden die Schulkinder für das Zeitalter in Anspruch genommen.'"

[3] In the Thaanith Treatise (fol. 276) the following passage occurs:

"...und die Standmänner (eine Vertretung des Volkes beim Kultus in Jerusalem) kamen in das Versammlungshaus und fasteten vier Tage, am 2., 3., 4., und 5. Wochentage, am 2. wegen der Seefahrer, am 3. wegen der Wuestereisenden, *am 4. wegen der Bräune* (*Askara*), *das sie nicht die Kinder befalle.*"

Its Eastern origin is also indicated by the statement of the celebrated Cappadocian physician Aretaeus (Mann, 1858), who lived in Rome at the end of the first century. Indeed, he was the first to give us an accurate account of pharyngeal diphtheria. After a minute account of the various forms of angina, the *Cynanche*, in which the inflamed tongue, owing to its excessive size, protrudes between the teeth, and the *Synanche*, in which the disease, hiding itself in the unseen parts of the chest, as it were clasps and torments the sick man inwardly, he describes in detail the diseases of the uvula, the *σταφυλή*, the *ἱμάντιον* and the *κράσπεδον* and (in the ninth chapter) the ulcers of the tonsils.

> "Ulcers sometimes arise on the tonsils, of which one kind is common, benign, and innocuous, the other rare, malignant and fatal. The benign ulcers are clean, small, shallow, uninflamed and painless; the malignant forms on the other hand are broad, deep, dirty, and covered with a white, bluish or black membrane. The latter are termed Aphthae, except when the disease extends more deeply, when it is called an Eschara. At the commencement of the Eschara there appears a vivid redness, and inflammation and pain in the veins as in carbuncles; then isolated pustules arise, to which new ones are continually added. These pustules coalesce and give rise to a broad ulcer. If the disease extends into the buccal cavity it attacks the uvula and destroys it, and then passes on to the gums, the tongue, and the angles of the mouth. The teeth are loosened and become black, and inflammation appears in the neck. Such patients die in a few days from the inflammation, fever, foul smell, and defective nutrition. When, however, the disease extends through the trachea into the thoracic organs death occurs on the same day by suffocation, for the lungs and heart can endure neither the smell nor the ulceration nor the pus, which excites coughing and dyspnoea.
>
> This disease may be produced by eating cold, rough, sharp, astringent things.... Hence it occurs most commonly in *children* up to the age of puberty, as these generally inhale much cold air, because they have the greatest warmth....As regards its local distribution this disease is most commonly found in *Egypt*, because in this country the air is dry for breathing and the food is varied, consisting of roots, herbs, many vegetables, pungent seeds, and thick beverages, either Nile water or sharp beer. The disease is very common too in *Coele Syria*. Hence the ulcers are often called *Egyptian* or *Syrian ulcers*."

This description by Aretaeus does not quite agree with the symptoms of diphtheria. It is remarkable that he considers these malignant ulcers to be rare, and describes no epidemics. The disease seems therefore to have occurred at that time only sporadically as an importation from Egypt and Syria, and never appears to have attained epidemic dimensions. The remarks of Aretaeus on the treatment of this disease are of great interest ("Curative treatment of malignant diseases of the throat" in the ninth chapter of the first book on the treatment of acute diseases).

> "The treatment of these diseases is in many respects similar to that which we have given for the treatment of diseases of the tonsils. Other special methods must, however, be employed. Clysters, venesection, embrocations, cataplasms, fomentations, bandages and cupping are used in such cases, just as in inflammation of the tonsils where we have the possible danger of choking. The local applications must however be more powerful, because the ulcers do not remain circumscribed and no scab is formed on the surface, and the sanious inwardly flowing pus continually eats deeper and rapidly destroys the still uninjured parts. The cautery might be employed, but owing to the narrowness of the parts its application would be attended with risk. We may however employ substances which in their action resemble fire in order to arrest the extension of the ulcers and promote the casting off of the membrane. Alum and honey, oak-gall and pomegranate blossom, and honey mixture are suitable for this purpose. These substances may either be blown onto the parts by means of a tube or applied to the ulcers by means of a long thick rod or feather. A good application consists of calcinated sulphate of iron with powdered tutty and vinegar. Two parts of moistened rhubarb root with one part of tutty may also be used. Care must be taken to avoid any pressure on the ulcers, because they are then apt to moisten and eat further. The dry medicaments should therefore be spread by means of a feather and the fluid preparations poured onto the uvula. When the crusts become loosened and the ulcers red there is great danger of convulsions arising, because the ulcers dry rapidly and thereby cause tension on the nerves. In such case we ought to moisten the parts and keep them moist by means of milk mixed with starch, barley water, or tragus juice or with linseed or hartshorn seeds. In certain cases the uvula is destroyed up to the bony palate, and the tonsils down to their bases, and the epiglottis is eaten away. On account of the scar thus produced the sick person is unable either to swallow dry or moist substances, and even fluids are rejected. Consequently the patient dies of hunger."

Almost all the later writers copy Aretaeus.

The description given by Aetius (1542) of Amida in Mesopotamia, who lived in the middle of the sixth century at the court of Justinian at Byzantium, is a little more characteristic. In the 46th chapter, "de crustosis et pestilentibus tonsillarum ulceribus," of the fourth part of the second book he writes:

> "Crustosa et pestilentia tonsillarum ulcera ut plurimum nullo praecedente tonsillarum fluxu incipiunt; aliquando autem a consuetis fieri inflammationibus, maxime efferatis, perficiuntur. Fiunt autem frequentissime pueris atque etiam aetate jam perfectis, maxime his qui vitiosis humoribus abundant, in iis quae vere contingere solent pestilentibus constitutionibus. In pueris vero ulcere, quod aphtham vocant, praecedente omnino perficiuntur. Sunt autem partim alba, maculis similia, partim cinereo colore, aut similia crustis, quae ferro inuruntur."

He warns us against violent removal of the crust and advises a careful application of the medicaments: "conandum autem est in universum ne digitum quidem locis admoveamus, aut leni saltem tractu manum admoliamur. Etenim inscii ad quos maxime in rebus dubiis homines confugiunt, vehementius illinunt, simulque inflammatum locum comprimunt, simulque crustam detrahunt: quod minime facere convenit prius quam elevatam, et vix innitentem crustam conspiciamus. Quod si enim adhaerentem adhuc crustam avellere aggrediamur, ulcerationes magis in profundum procedunt et inflammationes consequuntur, augenturque dolores, et in ulcera serpentia proficiunt. Itaque sicca quidem remedia insufflare convenit: liquida vero cum pinnula illinere; ita ut quantum licuerit quam penitissime pinnulam immittamus."

(He further says:) "Et febrium quoque curam habere convenit (vehementes enim incidere solent) atque in repurgandis explanandisque ulceribus maxime sollicitum esse; convulsionem enim infantes plurimi passi sunt in ulcerum repurgatione; aliqui vero via transglutiendi exsiccata, sunt strangulati. Sunt etiam quibus corroduntur gurguliones, atque ubi diutius perstiterint ulcerationes et in profundum proserpserint, cicatrici jam inducta, adstrictiorem vocem edunt, *reflectiturque ipsis potus in ipsas nares*; nam et ego puellam novi, quae post quadragesimum tandem diem consumpta est, quum se jam a morbo recolligeret. Verum ad septimum usque diem plurimi periclitantur."

Aetius was therefore acquainted with paralysis of the palate, a frequent sequel of diphtheria. As to treatment he recommends a series of astringent plant juices mixed with honey, and more especially the application of the dung of dogs which had previously been fed upon bones, or the excreta of boys who two days before had been fed upon bread and lupins, or in desperate cases dusting with dried and powdered crocodile bile or anointing with honey.

The earliest accounts of epidemics of diphtheria in Western countries date from the sixth century. Hirsch (1886) informs us that in the chronicle of St Denis of the year 580 a pestilence is mentioned, to which the name "Esquinancie" was given (corresponding to the term "Squinantia" subsequently applied to angina maligna). Hirsch further states that Baronius mentions a throat pestilence at Rome in the year 856, and another similar plague in the year 1004, which he describes as "catarrhus descendens in fauces meatus obstruens suffocatos miseros homines confestim mori cogebat." He also states that Cedrenus[1] records a plague, called by him *Cynanche*, which prevailed in certain provinces of the Byzantine kingdom in the year 1039 and caused great mortality, and that Short (1799) mentioned a kind of angina which occurred in England in 1389 and carried off a great number of children. He also refers to the accounts given by Frank von Wörd, who states that an unknown plague became epidemic in the Rhine region, in the year 1517, which produced a condition in which the "tongue and throat, covered as by a fungus, became white," and the patients could neither eat nor drink. These symptoms were accompanied by headache and fever.

---

[1] See Bibliography.

The same disease is also mentioned by Forestus (1588):

> "Aliter se habuit dolor gutturis, tum inflammatio in illa angina Epidemia, imo pestifera, et adeo maligna, et contagiosa, quae tempore M. Johannis Tyengii Amstelodamensis medici celebrioris in multos circa principium anni 1517 grassabatur, ut quibus intra sex aut octo horas remedia non adhibebantur, ante sedecim vel viginti horas, subito moriebantur; neque aliquis evadebat (ut perhibetur in ejus libello, propria manu scripto) si medico docto non uteretur...."

In the year 1557 he observed an epidemic in Alcmaria, "gutturis morbum," which affected whole families, and caused the death of over 200 people in a fortnight. This disease had, however, nothing in common with angina, as there were present only pains in the neck but no membranes. Sennertus (1628) mentions an epidemic of feverish angina in most districts of lower Germany and along the Rhine, which carried off many persons. van Wier (1660) gives an account of a deadly epidemic which broke out in the year 1564 in the same districts, spreading chiefly among children. Many died on the first day of the disease, but some lingered on till the third or fourth day, and a few even to the seventh. In these districts of lower Germany and in Holland the disease since that time appears to have become endemic.

The Epidemic of the Sixteenth Century in Spain and the subsequent Extension of the Disease.

In the second half of the 16th century, about the year 1581, there began in Spain a mighty epidemic which, under the name of "Garotillo" or morbus suffocans, has become well known. This epidemic, which undoubtedly was an outbreak of true diphtheria, spread up to the year 1618 over the whole of the Iberian peninsula and the neighbouring islands, and later over the whole of Italy. We have detailed reports from a great number of Spanish and Italian physicians upon this formidable outbreak. de Fontecha (1611), Villa Real (1611), and Herrera (1615) described it in Spain, and Alaymus (1632) and Cortesius (1625) in Italy. All of them mention the characteristic white deposits, later becoming discoloured, extending over the whole of the pharyngeal surfaces. Villa Real, especially, describes the false membranes as being so tough and elastic that they could be stretched like moist leather or parchment without tearing. Alaymus points out that the disease usually begins on the tonsils, uvula or throat, but also may arise in the larynx or occasionally in the nose. Death from choking was often observed. A horrible smell from the mouth was also commonly present, and was attributed to the affected parts becoming gangrenous. When this condition set in death usually took place between the fourth and seventh days. Cortesius was convinced that the disease was infectious, because many members of the same family became infected. The name "Gulae morbus" was given to the disease. After its appearance in Naples in the year 1642 the disease died out, and did not appear again until the end of the 17th century. A great extension however took place in the early part of the 18th century (Hirsch, 1886). Breaking out in the year 1701 in the Ionian Islands it spread into Italy, and in the year 1715 broke out again in Spain, and raged there in numerous epidemics, generally interrupted by long intervals. About the middle of the 18th century it became epidemic in France. In the year 1747 it extended into Italy, where it was carefully observed by Ghisi (1749) and described by him under the name "Angina strepitosa perfida mortalis." From France it extended into Holland.

Gerard van Swieten (1745), the celebrated commentator on Boerhaave's Aphorisms, was undoubtedly acquainted with the disease and even treated it successfully with spiritus salis marini (hydrochloric acid). In his work on angina inflammatoria he writes as follows:

> "Est et anginae species palatum, tonsillas occupans, quam frequenter observavi; quae facile curari plerumque solet, licet satis acriter saepe doleant partes. Inprimis in scorbuto laborantibus observatur, quandoque etiam epidemicorum more plurimos homines occupat, verno praecipue tempore, humidoque et calido aere. Levis febris praecedit, sed post aliquot horas evanida; tuncque dolent fauces hoc illove loco, et illas inspicienti apparet macula alba, cujus contigui limites rubent admodum et pessime dolent; caeterum non adeo intumescunt partes affectae. Si negligatur hoc malum, proserpunt hae maculae, halitus putridus ore exit, et eroduntur satis saepe profunde partes affectae. Spiritus sulphuris per campanam multa dilutus aqua, addito rob sambuci et nitro, curat felicissime tales anginas, si saepius de die ore continetur. Si jam putridum halitum expirent aegri, et profundius erodi inceperint partes, spiritus salis marini guttae triginta vel quadraginta, unciae uni mellis rosarum mistae, dant remedium, quod illinitum ilico sistit serpens malum: prodest tunc si sempervivi majoris succus cum melle vel syrupo althaeae mistus ore contineatur; postquam spiritus salis marini applicatus fuerit, sic enim dolor lenitur pulcherrime. Augetur jam vel minuitur spiritus salis marini quantitas pro vario natae putredinis gradu...."

Eventually the pestilence reached England, where it was minutely described by Fothergill (1748) as the "sore throat with ulcers," and also by Starr (1752), Huxham (1751—1753) and others. About the same time it spread over Switzerland, Germany, and Sweden. Simultaneously it was carried to America, and was already epidemic in New York in 1771-2. In 1779, according to Caldwell (1816), Washington was seized by the disease at his country seat, Mount Vernon, near Alexandria. It spread southwards as far as Jamaica.

Towards the end of the 18th century the disease once more completely died out in Europe. During the great pandemic in the middle of the 18th century a phenomenon was observed which had hitherto escaped notice, namely, the formation of tubular false membranes in the larynx and trachea. Ballonius (1762) was the first to describe such false membranes in the trachea. In his second book of epidemics and ephemerides he relates (p. 148) that three children died of dyspnoea, and from its symptoms he called the disease "affectio orthopnoica." "Dyspnoe erat et obaudibatur strepitus magnus cum raucedine." He points out that this phenomenon could be attributed neither to a catarrhus suffocans nor to a failure of the lungs. In one case he regarded the phenomenon as an "Indicium adventantis rubiolae," and then continues:

> "alius puer annos 7 natus consimili morbo interiit: non est deprehensa tam saevi morbi et difficultatis spirandi causa. Eo tempore vagabantur tusses, vulgo quintes dictae, de quibus paullo post. Et quod ex intervallis facit quintae tussis causa fugax, id facit eadem causa fixa: hic perseverat difficultas spirandi usque ad interitum. Filius D. le Noir ista difficultate spirandi interiit, quum raucedinem quandam haberet caninam, et tumentes paullum fauces: pulmo erat parte dextra vitiosa. Gervasius Honoré socer meus, ita paene suffocatus interiit. Chirurgus affirmat, se secuisse cadaver pueri ista difficili spiratione et morbo (ut dixi) incognito sublati; inventa est pituita lenta, contumax, quae instar membranae cujusdam arteriae asperae erat obtenta, ut non esset liber exitus et introitus spiritui externo; sic suffocatio repentina."

Ballonius adds that the disease from which the three children died might possibly be akin to the affection which Hippocrates termed "pulmo repletus." In any case it is clear from his description that he regarded the disease from which these children died as a new one.

It is noteworthy that in the 17th century, if we except certain statements by Ch. Bennet (1665), Nicolaus Tulpius (1685), Harris (cited by Fischer, 1830) and Struve (*ibid.*), phenomena of this kind had not been observed. It is not until the 18th century that we find the tracheal form of the disease described together with the pharyngeal form by Ghisi (1749) under the name of Angina strepitosa perfida mortalis. Ghisi observed that nasal voice and regurgitation of food through the nose were common sequelae of the tracheal form of the disease, and came to the conclusion that the two types were related. In France, epidemics of angina were observed which were characterised, especially in children, by the coughing up of tube-shaped false membranes from the trachea. Instances of this occurrence are noted by Arnaud de Nobleville (1748) in 1747 at Orleans and by Malouin (1746—47) in Paris. Starr (1752) in England also described and figured such false membranes as products of a disease which he called "morbus strangulatorius." Similar observations were made by Middleton (1756) in New York, Langhans (1753) in Switzerland, Nil Rosen von Rosenstein (1798) in Sweden, and by van Bergen in Frankfort, where an epidemic occurred in 1758. van Bergen called the disease "morbus truculentus infantum," and described the false membranes in the following terms:

> "Tussi rejecit tubulum membranaceum, qui portio membranae tubulosae per ramos bronchiorum durante morbo generata fuit."

In the year 1758 a severe epidemic occurred in Barbados, which was described by Hillary (1766) under the name "Catarrhus suffocativus."

In 1765 Francis Home published in Edinburgh a small work entitled *An enquiry into the nature, cause, and cure of croup*, in which, as the result of the observation of twelve cases, he describes a new disease. This disease, known locally on the west coast of England as "Chock" or "Stuffing," was designated by Home from its most striking symptoms, shrill voice and difficult breathing, "suffocatio stridula." According to Home it is especially common in children, the youngest being the most liable to it. He never observed a case in a child over twelve years of age, and never heard of one. He considered damp, cold weather in the winter months, and the neighbourhood of the sea, as the chief predisposing factors. His descriptions include two forms, a benign purely catarrhal form, and a malignant form, characterised by the formation of false membrane usually in the upper part of the trachea, from which it spread downwards. This membrane was often so tough that it could be soaked in warm water for as long as four days without being dissolved. It was not attached to the underlying parts, and could be easily separated from them because there was pus behind it. Where it ceased the trachea was covered with pus or purulent mucus. Home particularly points out that the new disease, at least in Edinburgh, was so rarely observed that a single individual might during the whole course of his life only come across a few cases. As to treatment he recommends venesection and the local application of leeches, the use of blisters round the neck, attention to good evacuations, and the inspiration of steam and alcohol vapour. He saw no special utility in sudorific substances or in emetics. When the membrane was once formed the only thing was to try to remove it, and for this purpose he recommended, in certain cases, tracheotomy. Home's publication aroused the attention of the whole medical world. Everywhere the new disease was actively studied, and Home's statements were repeatedly confirmed. While, like Home, some observers only met with sporadic cases, others described epidemics. Amongst the latter were Lepécq de la Clôture (1778) in the department of the Lower Seine, and Ramsey (about 1786) at Chesham. As in the earlier epidemics cases corresponding to Home's type were frequently observed, together with angina gangrenosa maligna. Samuel Bard (1789), Johnstone (1779) and others consequently regarded these conditions as related to one another.

The difficulty of differentiating these diseases was rendered still greater by the fact that John Millar (1769) had described, under the name of "Asthma acutum," a disease very similar to croup in its clinical symptoms, but differing from it in the absence of cough, the rattling rather than whistling tone of the respiration, and absence of membrane. In this condition the question remained for more than a hundred years, until the discovery of the causal agent of diphtheria finally enabled us to classify these diseases on a satisfactory basis.

The influence exerted by Home's book is seen from the fact that the Société Royale de Médecine of Paris in 1785 offered a prize on the subject "Si la maladie connue en Écosse et en Suède sous le nom de croup ou angine membraneuse existe en France?" Vieusseux (1812) in the paper which obtained the prize stated that croup had long been known in Geneva, and that it probably existed also in France. The essays of the other competitors clearly demonstrated its presence in France. These works advanced our knowledge of diphtheria as little as the subsequent descriptions of certain epidemics of croup at the beginning of the 19th century. One fact alone appeared to be clearly established, namely, that croup was becoming less frequent.

Special interest was again directed to croup when the favourite nephew of Napoleon the First, the son of Louis Bonaparte, King of Holland, died from it. In consequence of this, Napoleon, on the fourth of June 1807, again offered a prize for the best essay on the subject. Seventy-nine essays were presented and carefully criticised by Royer-Collard (1813), who awarded prizes to Albers (1816) and Jurine (1816) The observations of Jurine on the relation of croup to angina gangrenosa are specially interesting. "Gangrenous angina, Cynanche maligna, or angina gangrenosa is a disease which occurs in a pharyngeal, laryngeal or tracheal form. In the latter form it might easily be confused with croup, one of whose symptoms, namely the false membrane, it exhibits if it did not at the same time possess other distinguishing characteristics." After describing the course of gangrenous angina and the differential diagnosis from croup he continues: "There is, however, still another, perhaps also infectious, disease with which croup is often confused which deserves the attention of physicians, since in a certain number of cases it resembles gangrenous angina, I mean the gangrenous angina of children. If we read the reports of writers who have described this disease and take into account the predisposition of children to it, the rapidity with which the membrane forms, and the condition of the spots or ulcers on the tonsils and pharyngeal region, we are tempted to doubt the existence of gangrene as a specific disease in the majority of these cases, and to regard the disease as modified croup, which has merely assumed another form owing to the putrid influence of the epidemic, and to term it aphthous, putrid or malignant croup." He also denies that croup is infectious, stating that " the view taken by certain writers that croup is infectious is probably based on the fact that it is epidemic." In another place he says that the infectious nature of croup is inferred from the fact that outbreaks occur in relation to epidemics of gangrenous angina, German measles, small-pox, varicella and scarlet fever. In these instances he considers the croup as an epiphenomenon superadded to the disease. These prize essays gave very little information as to the cause of croup. Jurine considered that a checking of the perspiration by the action of cold air was the chief cause. He attributed the severe outbreak of croup among northern nations to a change in their mode of life and to the relaxation brought about by it.

Owing to the demand for suitable treatment, the question whether croup was an inflammatory affection or not was the subject mainly discussed in these essays. Some maintained that they had always found inflammation of the mucous membranes of the larynx, while others denied this. Lobstein (1817) inclined to the latter view, and held that in croup we have not a particular condition of the blood but a catarrh with which is associated a nervous element. The false membrane is not the essential element in croup, since the sick person often dies, although the membrane has been removed and the air-passages rendered free. Autenrieth (1807) had previously made similar observations which led him to a peculiar conception of the cause of the disease. He regarded it as probable that

> "Der Grund der Epidemie der Luftröhrenentzündung in einer Mischungs-Veränderung des Körpers lag, welche langsam vorbereitet wurde, aber durch äussere Umstände schnell vollendet, gleichsam auf einmal den Stoss zum Ausbruch erhielt; dass diese Mischungsänderung in Zersetzung eines Teiles der Säftemasse bestand, und nun ein reizendes Produkt sich entwickelte, was, weil es nicht in fester Form sich darstellte, mittelst des Kreislaufes bald auf dieses, bald auf jenes Organ durch das Bestreben des Körpers, die unzweckmässige Mischung auszustossen, geworfen werden konnte. Ein Produkt, das bei der rheumatisch-gastrischen Grundform des epidemischen Charakters vorbereitet geworden zu sein scheint; indem es durch Hemmung der Hautausdünstung, und darauf folgenden unzureichenden Versuch der gastrischen Organe, die Funktion der Haut vicarierend zu ersetzen, entstand, nun durch Witterungsumstände auf die Respirationsorgane gelockt, daselbst durch höhere Oxydation ätzender gemacht wurde; und bei der Anlage zur Entzündung dieser Teile, die durch eben diese auch die Arteriosität und das Leben der kleinsten Gefäschen bis zum Ueberreizen vermehrende Oxydation sich bildete, jetzt die Reizbarkeit des Körpers von jenen Organen aus, bis zur Zerstörung aufregte und erschöpfte." From this hypothesis he concludes that "nur das Mittel in der Luftröhrenentzündung allen Forderungen der Kunst entsprechen wird, das zugleich die Natur der pathischen Schärfe umändert, die Erregung herabstimmt, die Möglichkeit überwiegender Tätigkeit der gastrischen Organe herbei führt, diese Organe selbst reizt, und so die Wiederherstellung des Gleichgewichts im Körper, durch critische Ausscheidungen möglich macht; ein Mittel also, das den ersten Grund der Krankheit selbst tilgt, und damit gleich gut sowohl in der Form einseitiger Nerventätigkeit, als übertriebener Gefässtätigkeit hilft. Dieses Mittel bietet der innerliche Gebrauch der Quecksilberkalke oder der milden Verbindungen des Quecksilbers mit Säuren dar."

Besides applications of mercury he first used vinegar clysters in order to drive the morbid material towards the stomach, and later cutaneous irritants to drive it towards the surface of the body, because under these conditions the "concentration of irritability towards the air-passages will cease of itself." He did not therefore trouble himself much about the pathological products in the throat, and even thought local treatment to be injurious because it increased the irritability of these parts. For the same reason he denied the advisability of tracheotomy. The statements of the keen-sighted Tübingen professor upon the debated question whether membranous angina was an independent disease or merely an intense form of catarrh are of particular interest. He considered that

> "Der ganze Verlauf der Epidemie zeigte nämlich, das sie zwar entsprungen war aus einer erhöhten catarrhalischen Affektion, aber in ihrer Ausbildung eine Selbstständigkeit ihrer Symptome erhalten hatte, welche bei der Abnahme der Epidemie nicht wieder zum Catarrh zurückkehrten, sondern charakteristisch bleibend, aber immer schwächer werdend, endlich erloschen. Es gehört zum Beweise der Verschiedenheit zwischen der catarrhalischen Basis der Krankheit und der

entwickelten, gleichsam erst in der Folge selbstständig gewordenen Luftröhrenentzündung, dass Kinder, welche schon früher im Winter die unten näher zu beschreibende unbedeutendere rheumatische Krankheit gehabt hatten, doch späterhin nicht verschont bleiben von der heftigeren Krankheit. Während folgendes noch mehr beweist, das die Luftröhrenentzündung einmal gebildet, eine bestimmte Krankheit, nicht blos ein höherer Grad eines gewöhnlichen Catarrhs wird, der einen und denselbigen Menschen vielmal in seinem Leben befallen kann; nämlich *einmal heftig von der Luftröhrenentzündung befallene und gerettete Kinder, scheinen frei davon für die Zukunft zu sein*, während Kinder, welche einige Jahre vorher schon schnelle, aber nicht heftige Anfälle, vorzügliche der krampftgen Form der Luftröhrenentzündung, gehabt hatten, dieses Jahr teils nicht frei blieben von Anfällen der nämlichen Krankheit, wenn sie gleich nur einen geringen, kurze Zeit dauernden Anfall wieder erlitten. Man könnte von diesen Kindern sagen, sie befiel in zwei verschiedenen Zeiten die nämliche Krankheit, aber in zwei Hälften geteilt, jedesmal also mit desto geringerer Stärke; die Receptivität, welche der erste Anfall nicht gänzlich erschöpfen konnte, erschöpfte der zweite. *Bei allen selbstständigen Krankheiten, die nicht als blos höherer Grad einer andern Krankheit angesehen werden können, findet das nämliche statt*; so bei dem Krampfhusten, bei den Masern, welche beide Krankheiten zu der nämlichen natürlichen Familie mit der Luftröhrenentzündung gehören. Selbst bei den Pocken findet etwas ähnliches statt. *Alle diese Krankheiten befallen den Menschen in seinem Leben nur einmal*, wenn nicht der zu schwache erste Anfall noch Receptivität für einen zweiten oft lange erst nachher kommenden übrig liess. Bei einigen Krankheiten der Tiere, auffallend z. B. bei der Rinderpest, ist es derselbige Fall. Gehört aber gleich die Luftröhrenentzündung hierin zu diesen ansteckenden Krankheiten, so dürfte diese Aehnlichkeit doch noch kein vollgültiger Beweis für ihre Ansteckungsfähigkeit sein. Es könnte bei ihr (ohne dass den Individuen einer Tierspecies gleich, ein durch die Reihe von Zeugungen und Erzeugtsein zusammenhängendes, materielles Contagium alle einzelnen Fälle verknüpft hätte), eine fortschreitende Veränderung der in der Atmosphäre und im Körper liegenden Bedingungen zur Erzeugung der Krankheit, in jedem Einzelnen mit vorrückendem Frühjahr die gleiche Veränderungen erzeugt haben; und so die Entfernung der Krankheitsform von der gewöhnlichen catarrhalischen, gleichsam das nun selbstständig und abgeschnitten Charakteristische derselbigen gebildet haben. Umgekehrt erweist die, weniger Zweifel ausgesetzte Ansteckungsfähigkeit der Masern noch nicht, das nicht diese Krankheit, oft auch bei uns, langsam aus einer catarrhalischen Basis sich entwickelt, und Selbstständigkeit erst bei einer gewissen Stufe der Entwicklung der Epidemie erhält."

We see, therefore, that Autenrieth really recognised diphtheria to be an independent disease conferring immunity after recovery and depending on a pathological agent circulating in the blood; but, fettered as he was by the views of his time upon the epidemic Constellation and Disposition, he was unable to acknowledge frankly its infective nature. He declared that membranous angina and Millar's asthma were identical, but gave no indication as to their relationship to angina maligna.

Virulent outbreaks of the disease between the years 1818–1826 in Tours, La Ferrière and Chenusson, comparable with the notorious "Garrotillo" in Spain, provided Bretonneau (1826) with the material for his admirable investigations. In the year 1818 the garrison of Bourbon-Vendée was transferred to Tours, and many fell ill there of a disease characterised by ulcers in the mouth, inflammation of the gums, and breaking of the teeth followed by grey-green deposits upon the mucous membranes of the lips and cheeks. From the mouths of the sick persons there emanated a pestilential odour, and the neighbouring lymphatic glands began to swell. The disease was at first regarded as scorbutic gangrene of the mouth, but Bretonneau soon recognised that it could have no connection with scurvy, because the persons attacked were in other respects healthy, and also because it assumed all the features of angina maligna when it attacked the tonsils and throat. Bretonneau attributed the striking fact that it primarily attacked the gums to the use of drinking vessels in common. When the garrison was replaced after a time by another section of troops the disease appeared among the latter in the form of severe angina maligna. Within a few months of the introduction of the disease by the soldiers from La Vendée to Tours 60 persons of all ages, mostly however children, died from it. Some fell ill of severe gangrenous angina, others of typical croup. An accurate clinical and pathologico-anatomical investigation of all these cases led Bretonneau to the conviction that all of them were caused by one and the same disease, to which he gave the name "diphthérite" (*ἡ διφθέρα*, the skin) in order clearly to distinguish it. The production of membrane by the action of the virus was to him the characteristic mark of the disease, because in the severe, apparently gangrenous, forms he found at the autopsy not the expected gangrene of the mucous surfaces, but a *membrane* of a greyish-green colour, owing to the decomposition of the blood mixed with it, lying upon the slightly altered tissues. This was the source of the pestilential odour which had suggested a gangrenous condition. The association of malignant angina with typical croup, which he had observed in many cases, at first led him to think that the condition might be due to the occurrence of two diseases simultaneously, but the observation that adults who were suffering from malignant angina could infect children with typical croup, and the fact that the deposits on the tonsils and pharynx exhibited the same structure as the membranes of croup, convinced him that croup and malignant angina *must be dependent on the same disease-producing factor.* He further states very decidedly that scarlet fever angina has nothing in common with diphtheritic angina, because the diphtheria membrane is formed upon the mucous membrane and can be detached, whereas in scarlet fever the change is in the mucous membrane itself and the deposit cannot be removed. The keen-sighted observations and deductions of Bretonneau led to the conclusion that diphtheritis, although occurring under different forms, is a single etiological ens morbi, just like scarlet fever, measles and small-pox. Trousseau (1828) confirmed and extended Bretonneau's fundamental investigations, but dissented from some of his deductions. He substituted the name "diphthérie" for "diphthérite" in order to indicate that he did not share the view held by Bretonneau of the purely local character of the disease, but looked upon the specific changes in the mucous membranes as the products of a general disease running its course in the patient. This name was subsequently accepted by Bretonneau himself, because the term diphtheritis was used by many in a sense different from that which he intended. He also spoke of the disease as Egyptian diphtheria in order to commemorate the land of its origin.

The Distribution of Diphtheria in the Nineteenth Century.

Before considering further observations on the etiology of diphtheria, which followed those of Bretonneau, it may be desirable to review (following Hirsch, 1886) the general spread of the disease in the 19th century. The disease reached its present extension in France in the years 1825–1836. At that time it also spread over Switzerland. In Germany isolated epidemics were observed on the Baltic coast, in East Prussia, and later in the Harz and in Lüneburg. Isolated epidemics were also reported in England, Norway and the United States. In the forties many epidemics were recorded in Germany, especially in Greifswald and Königsberg, in England, Holland, Belgium, France, Denmark, Norway, and North America, in fact, in almost every part of Western and Northern Europe and North America. For a few years the disease was less common, but in the middle of the fifties there began an extraordinary pandemic extension which spread over the whole civilised world, even reaching China, India, Australia, and Polynesia. France was again the starting point of the outbreak. In succession the Iberian peninsula, Holland, England, Germany, Russia, and America were invaded, and a little later Norway and Sweden, Italy and Austria, and in the seventies South Russia and Roumania. The victims in Bessarabia were numbered by hundreds in every village, and the children almost disappeared. In Iceland the disease appeared for the first time in the year 1856. Following some earlier epidemics (in the twenties) in the Argentine the disease spread over South America and became firmly established. It attacked North Africa, Tunis and Alexandria in the seventies. In Egypt, the land of its origin, its extension was limited, and in Syria the pestilence was said to have been unknown up to the year 1868. In Persia a severe outbreak occurred in the seventies. In India it appeared on the slopes of the Himalayas in the fifties, in Madras in the sixties, and on the Indian Islands at the commencement of the eighties. In China the disease is said to have become endemic in the year 1821. In the year 1866 a frightful epidemic occurred at Pekin, from which 25,000 people are said to have died. In Japan the disease first appeared in 1877, and has since become endemic. At the present time it is an endemic disease throughout the civilised world.

The sudden and apparently inexplicable outbreaks, which occur in regions from which the disease has temporarily disappeared, may be explained by the recent remarkable advances in means of communication which frequently lead to the reintroduction of the infective agent.

Opinions on the Nature of Diphtheria in the middle of the Nineteenth Century.

Bretonneau's statements were well received throughout France, but found less ready acceptance in England and Germany. The view that the local phenomena owed their origin primarily to the entrance of the infective agent was frequently disputed. The view, maintained by Trousseau, was generally held that the local lesions were manifestations of a general disease, and the fact that similar pathological changes were found in the larynx, pharynx and trachea in several infectious diseases strongly supported this view. Owing to the extraordinary influence of Virchow (1844) pathological anatomy during the forties began to occupy a prominent place in the scientific investigation of the processes of disease. These investigations showed that various forms of inflammation of the mucous membranes could be distinguished, namely, the simple catarrhal form which led to the formation of false membrane, and the form in which, together with necrosis, there was a deposition of fibrinous exudation in the mucous membrane itself, to which Virchow gave the unfortunate name "diphtherische." It was found that the development of these forms depended on the action of the exciting agent, and pathological anatomists consequently laid stress on the *form of the change in the mucous membrane.* Since the various forms were found in persons who had died both of Bretonneau's disease, diphtheria, and of other infectious diseases such as scarlet fever, measles, typhus, small-pox, etc., there was no reason to suppose that the croupous or pseudo-membranous deposits, which were so frequently found in diphtheria, were pathognomonic of that disease. From the point of view of the pathological anatomist, therefore, the etiological factor which Bretonneau had tried so hard to establish was thrown into the background. After the occurrence of widespread epidemics little doubt could be entertained that angina maligna was infectious, but the infectious nature of croup was not yet fully established. Wilhelm Sachse (1810) in his well-known work, *Das Wissenswürdigste über die häutige Bräune,* which appeared in Lübeck in the year 1810, carefully reviewed the opinions of previous writers on the latter question, and showed that at that time opinions were equally divided. Though Bretonneau's observation made in Tours produced convincing proofs of the infectious nature of all these forms of disease a long time elapsed before physicians accepted his views.

In his open letter to Blache and Guersant, which appeared in the year 1855, Bretonneau once more asserted his conviction that all forms of the disease were contagious, and deeply deplored the attempt which was being made to shake the belief in the transmissibility of the disease. He again quoted the celebrated case in Napoleon's family. Queen Hortense was stricken with a protracted diphtheritic inflammation of the gums, and her eldest son died from laryngeal diphtheria. Later her mother, the Empress Josephine, was seized with diphtheritic angina, and died several days later from an attack of croup. In this letter he makes some important observations on the mode of transmission, pointing out that the infective agent is not carried by air, and that direct inoculation upon the softened mucous membrane or upon the injured epidermis is necessary. In proof of these statements he adduced the records of numerous observers in small districts where every detail of the invasion of the disease, its seat, its transmission from one family to another, its conveyance to particular houses and to other localities, could be easily traced.

The transmissibility of the disease in the manner described by Bretonneau was, however, proved by numerous cases of illness amongst physicians and nurses attending on diphtheria patients who happened to be directly inoculated by the secretion. Special attention was aroused by the cases of Dr Villeix and Professor Herpin (1843) of the hospital at Tours. The latter, when cauterising the throat of a child suffering from diphtheria, was struck on one of his nostrils by membrane expelled during a fit of coughing. Occupied with the child he was unable to remove the membrane at once. Severe diphtheritic inflammation developed at this place and spread over the whole nasal cavity. Later severe paralysis occurred, from which he finally died. A significant case was also observed at the Collège de la Flèche, where a healthy child, treading with naked frost-bitten feet on material expectorated by a diphtheria patient, soon after fell ill with formation of membrane between the toes. Another interesting case was observed by Paterson (1866). In this instance a farmer, who had already lost two children from diphtheria, inserted his forefinger, on which there was an open wound, into the throat of a third child suffering from the same disease in order to remove an obstruction which had brought on a fit of coughing. A membranous layer developed on the wound and extended over nearly the whole finger, accompanied by severe general phenomena. After some weeks the finger healed. Although the mucous membrane of the throat had never been affected, several weeks later severe paralysis of the legs, and later of the arms, supervened, from which he only recovered after four months' treatment.

Experiments on Animals.

Against the numerous involuntary inoculations with diphtheritic material were advanced the negative results of direct inoculation. The well-known auto-inoculations of Trousseau (1828) and Peter (1859), the 20 prophylactic inoculations of Mosotto (1864) and the application of membrane to varicose ulcers by Felix (1870) were not followed by ill effects. Further, the experimental inoculation of the virus into animals did not lead to convincing results. Albers, Jurine, Duval, Schloepfer, Valentin (cited by Pauli, 1865) and others, starting from the conviction that croup was set up by the inspiration of acrid irritant substances, endeavoured by introducing acrid substances such as acids, alcohol, and lunar caustic into the air-passages to produce false membranes such as characterise croup. Their attempts, however, were unsuccessful. On the other hand Bretonneau succeeded in producing typical false membranes by injecting a mixture of cantharides and olive oil into the trachea of dogs. Still more striking results were produced by Delafond by introducing chlorine, corrosive sublimate, arsenic, sulphuric acid and ammonia. Ammonia was found to be the most suitable substance for such experiments, and was therefore made use of by later investigators, Reitz, Oertel and others. Bretonneau, however, had already clearly recognised that, in spite of the similarity of the changes produced by tincture of cantharides in dogs and diphtheria in man, the two kinds of inflammation were by no means identical, since they differed markedly in their course. Consequently he stated without hesitation that diphtheritic inflammations were *specifically* different from the apparently similar ones produced by chemical agents. He had previously endeavoured to produce artificially the lesions of diphtheria in animals by the inoculation of material derived from patients, but had not met with success. Although unsuccessful these experiments constituted a step in the right direction, and the observations of later investigators along similar lines produced interesting though inconclusive results.

Labadie-Lagrave (1873) inoculated subcutaneously perfectly fresh pieces of membrane obtained at a tracheotomy operation into three young rabbits, and the blood of a child which had died of diphtheria into the femoral vein of a rabbit, without producing any results. On the other hand, when he introduced fragments of false membrane into the tracheas of two rabbits false membranes developed, and the animals died within 12 hours. Duchamp (1858) obtained similar results. Intravenous and subcutaneous inoculations of false membranes into rabbits and horses produced no ill effects, whereas inoculations into the trachea of the rabbit led to the formation of false membrane. He did not, however, succeed in transferring the disease from rabbit to rabbit.

Homolle (1875) made numerous experiments without producing any phenomena comparable to those of human diphtheria. On the other hand Everett (1881) always succeeded, even when he introduced particles of membrane into the uninjured trachea. Francotte (1883) repeated these experiments in Vulpian's laboratory, introducing finely divided membranes into the opened tracheas of six rabbits. Only one of these animals exhibited extensive false membrane in the larynx and trachea. The animal also showed congestion of the kidneys and hepatisation of the upper lobes of both lungs. The membrane, when transferred into the trachea of another rabbit, produced no effect. In the other five he found catarrh of the air-passages and ecchymoses of the mucous membranes of the larynx and trachea, but no false membrane.

Similar investigations were also carried out in Germany. Hueter and Tommasi-Crudeli (1868) found that the inoculation of fragments of fresh membrane, obtained both from the trachea and the throat of diphtheritic patients, into the dorsal muscles of the rabbit caused death in 20-40 hours. At the point of inoculation they found small abscess cavities containing a few drops of pus, and immediately surrounding them a diphtheritic inflammation which gradually merged into an extensive haemorrhagic inflammation of the muscle substance. On further investigation they discovered that similar lesions could be produced by the inoculation of putrid substances. Trendelenburg (1869) introduced fragments of diphtheria membrane into the tracheas of rabbits and pigeons, and recorded 11 positive results (eight rabbits and three pigeons) in 68 experiments. Oertel (1871) also made a large number of similar experiments, in many instances with positive results. He found that while inoculation with pulped material was particularly successful, the introduction of compact elastic fragments of membrane did not cause any membrane formation in the trachea. In one series of experiments he succeeded in transferring the disease through a series of six animals (four rabbits and two pigeons), partly by tracheal and partly by intramuscular injections. Control experiments made with putrid substances never had the same action as the diphtheritic material. This fact was confirmed by Billroth (1870) in a series of similar experiments. Oertel therefore became firmly convinced that the inoculation disease produced by him was transmitted diphtheria. These experiments, however, lost much of their weight from the fact that other investigators, Hueter (1873) and Marcuse (1875), succeeded in experimentally producing similar false-membranous processes by the inoculation of non-diphtheritic material and putrid blood to those produced by the injection of pieces of diphtheria membrane. Marcuse, notwithstanding these experiments, still maintained the specific action of the diphtheria virus in the production of false membrane, arguing that anatomically and symptomatically analogous processes might be produced by agents entirely different from each other in their nature.

That Oertel's experiments had not decided the question is clear from the communication made by Lichtheim to the Second Congress for Internal Medicine in the year 1883. Lichtheim, after stating his conviction that the disease beginning in the neck and descending to the larynx is in a clinical sense a single disease, gave a report on some of his own inoculation experiments. His conclusions were that it was possible to produce two types of lesions, the inoculation disease described by Oertel, characterised by an oedematous infiltration of the tissues surrounding the site of inoculation and progressing along the lymph channels, and secondly, a much rarer form, characterised by a peculiar necrosis. He concluded that all these inoculation diseases had nothing to do with diphtheria, and that experiments on animals did not therefore throw any light on the essential nature of diphtheria.

The Rise of Bacteriology.

As these statements did not completely settle the matter, it became necessary to obtain a knowledge of the etiological factor in the disease, and this could only be obtained by bacteriological investigations. About 1840 the doctrine of the *contagium animatum* obtained a special ascendency. The discovery of Bassi (1837)[1], that a miasmatic contagious disease of the silk-worm, muscardine, was caused by a fungus, stimulated researches on these lines. Before this time several observers had noted various forms of moulds upon dead animals, insects and fish, and they assumed that these organisms had caused the death of the animals. All morbid changes on the surface of man and animals were investigated for the presence of any kind of fungus, and it was not long therefore before the membranes of croup were subjected to this kind of investigation. Bennet (about 1843) and Schaffner and Remak (1845) found in them many organisms belonging to vegetable types. Laycock, in a paper published in Edinburgh on the 29th of May 1859, declared that diphtheria was due to the development of *Oidium albicans*. John Lawe (1859) also reported that he had observed this fungus extending into the bronchi, and N. Jodin (1859) in Paris also considered it to be the cause of croup. These views were, however, questioned by Thomas Hillier (1859) and Julius Althaus (1860) on the ground that it was not *Oidium albicans* but *Leptothrix buccalis* which was found in false membranes, and that the presence of the latter in diphtheria was of no significance. In the year 1860 Desmartis and Bouché de Vitray sought to demonstrate that croup owed its origin to a vegetable parasite, *Leptotoia buccalis,* found in the throat. Jodin maintained that he could cure the disease by purely local treatment with tinctura ferri muriatici, which killed the fungus. Laboulbène in the year 1861 also called attention to the micro-organisms in the false membrane. He found: "parfois des végétaux sous forme de spores ou de mycélium et des vibrioniens des genres bacterium et vibrio, surtout du premier genre, ayant le corp filiforme et raide." These vibrios appeared to him "distincts des fragments brisés du leptothrix buccalis."

In the year 1867 Buhl gave an account of his researches in the following words:—"I refer to the fungus which constantly occurs in diphtheritis and grows into and permeates the epithelial layer. Its elements are so small that unskilled persons are frequently led to the erroneous conclusion that the object under examination represents a complete granular breaking down of the tissues. Whether this fungus is a particular species essential to diphtheria or is an ordinary *Leptothrix buccalis* occurring in the mucus of the mouth, and consequently merely an accidental saprophyte which has fallen on favourable soil, I leave undecided since the question is at present beyond my knowledge." In the same year Tigri (1867) made a communication to the Académie de Médecine upon "un cryptogame rameux articulé," which he considered to be the cause of diphtheria. The investigations of Hallier (1868) led him to conclude that *Diplosporium fuscum* was the cause of diphtheria. The influence exercised by Hallier led to the discovery of the *Zygodesmus fuscus*, and later the *Tilletia diphtheritica* of Letzerich (I. 1869).

---

[1] Bassi (1837), *Del mal del segno, calcinaccio e moscardino.* 2^d ed., Milano.

In the light of our present knowledge it is remarkable that, until recently, actual fungi were discovered and cultivated, and even successfully inoculated into animals and considered the cause of diphtheria. In the *Progrès médicale* of the year 1881 we find Talamon describing with the greatest minuteness a fungus, which he invariably obtained in his cultures, and with which he made inoculation experiments into rabbits, guinea-pigs, frogs, fowls and pigeons. In the pigeon especially he produced thick false membranes within the nasal cavity, and was able to recover the fungus by cultivation, both from the membranes and from the fluids of the body cavities. Cultures from the blood, however, remained sterile. He demonstrated the same fungus in preparations from thick membranes covering the pharyngeal and laryngeal passages of three young kittens. In his opinion the kittens were infected from the straw litter upon which four of the rabbits, inoculated by him with a culture of the fungus, had died. In France especially these statements at first aroused much interest, but were soon criticised. Duclaux (1882) pointed out that the fungus had no special relation to the diseased parts, and that the methods of cultivation were not described with sufficient accuracy to allow others to repeat the experiments.

About 1870 many experiments were made with the object of ascertaining the relationship of the bacteria to diphtheria. At that period almost all investigators were influenced by Hallier's publications in which he strove to demonstrate that the smallest forms of life, the cocci and bacteria, were the lowest developmental stages of the more highly organised moulds. In 1868 Hueter and Tommasi-Crudeli (1868) found in the blood of diphtheria patients small rounded motile bodies which they regarded as vegetable organisms. They also found these bodies in the membrane and in the lesions which they produced in animals by the inoculation of pieces of membrane. Hueter, following the example of Hallier, made cultures by sowing pieces of membrane from the trachea and bronchi of a child upon cooked potatoes. Professor Hoffmann, of Giessen, found on three preparations dark yellow, orange yellow and violet slime, which under the microscope consisted of small cocci and rods. Hueter therefore believed that he had proved the vegetable nature of the diphtheria virus. Oertel (1868) confirmed some of these facts. He found micrococci not only in the membranes, but also in the lymphatic vessels of the submucosa, in the organs, especially the kidneys, and in immense masses in the form of colonies on the pavement epithelium of the pharynx. Oertel, correctly estimating the position of bacterial investigations at that time, abstained from making culture experiments because, as he wrote in 1871, "in the first place there are a series of questions of vital importance that still remain to be solved. The various yeast forms, the putrefactive yeast, the micrococcus and the schizomycetes, and their connection with definite forms of fungi or their independent individuality and the conditions of their vegetative processes must first be investigated, before it can be possible by the inoculation of any kind of spores to institute an exact pathological experiment." Nassiloff (1870), too, found in diphtheritic material accumulations of granules, and with them vibrios, which were identical in appearance with the organisms which appeared during the processes occurring in the putrefaction of normal blood and muscle. These he inoculated into the cornea of the rabbit. Working with the putrefactive organisms he was unable to produce any inflammation, but succeeded in doing so with the other organisms. He found the fungi both in and on the epithelium, either singly or in flat masses, or grouped together into sharply defined balls, penetrating inwards in the form of irregular bands. He could also demonstrate in the vicinity a reactive cell-proliferation, and came to the conclusion that in the development of the organism we had the starting point for diphtheritic changes.

Von Recklingshausen (1871), again, in a whole series of infective diseases, pyaemia, puerperal fever, typhus, acute splenic inflammations, urine infiltrations and pulmonary gangrene, found the cause of the metastatic foci to be aggregations of minute organisms, which he regarded as identical with the forms described in diphtheria by Buhl, Oertel and Nassiloff. Eberth (1872) made transverse sections through small island-like deposits on the surface of the mucous membrane, and found them to consist of masses of densely crowded punctiform shining spores in a homogeneous somewhat tough ground substance. In addition he found young cells in varying numbers, and in the deeper parts a network of coarser and finer trabeculae of fibrin. The epithelium of the mucous membrane was generally still present below the deposits, but permeated and rendered cloudy by young cells. By the use of acetic acid and caustic soda, the cloudiness was shown to be due to numerous shining punctiform corpuscles, the spores, lying between and in the epithelial cells. He found also micrococci in star- and spindle-shaped groups and rounded clumps filling up the dilated lymph canals of the mucous membrane and of the adventitia of the vessels. During the more advanced stages the tissues were permeated by innumerable masses of fungi. In his investigations he never met with bacteria in the blood, liver, or kidneys of persons who had died from pharyngeal diphtheria. By the inoculation of the micrococci into the cornea of the rabbit he obtained the same results as Nassiloff. In further experiments (1873) he found that the globular bacteria of the oral cavity and the micrococci cultivated in putrid flesh and putrid urine set up lesions analogous to diphtheritic processes, only differing in their severity. Meanwhile Leber (1873), Stromeyer (1873), and especially Dolschenkow (1873) obtained, by the inoculation of putrid substances, severe lesions in the cornea, such as hypopyon-keratitis, and even panophthalmitis. The discovery of bacteria in the membranes was confirmed by many investigators, and interpreted by some as essential, by others as accidental. Others however failed to find them. Nothing of special importance was added to our knowledge by the investigations of Classen (1871), Senator (1872), Weigert (1877), Satterthwaite and Curtis (1876), Leloir (1880), Cornil (1881), and Everett (1881), nor by the investigations of the Commission of the Royal Medical and Chirurgical Society (1879) upon the question of the occurrence of a non-diphtheritic laryngitis.

The investigations of Letzerich (1880), however, are of peculiar interest, since he was the first to cultivate diphtheritic material in "Gallertkammern[1]." In these he observed the development of the *Micrococcus diphtheriticus* in the so-called plasma cells, which he looked upon as the mother-cells of the micrococcus colonies, and also the emigration of the micrococci from the latter. These plasma cells he found in large numbers along the walls of the heart veins, in the lumen of the renal tubules and in the lymph-spaces of the lymphatic glands. In opposition to Letzerich, Klebs (1881) found no micro-organisms in the internal organs of diphtheria patients, but found them in large numbers in the membranes. He also made cultivation experiments obtaining the material by introducing fine capillary tubes into the tonsillar deposit of a child which had died from diphtheria. The contents of these tubes were sown on round masses or thin layers of gelatin in von Recklingshausen's shallow glass dishes. In about five days numbers of intensely brown granules and round dull coloured discs developed, the largest of which scarcely attained the size of red blood corpuscles. In three weeks the medium became liquid and contained innumerable, extremely small bacilli. Letzerich and Klebs were therefore the first to cultivate diphtheritic material on solid nutrient media. The structures described by Klebs as plasma-spheres, brown balls and pale discs were nothing else than colonies of various kinds of bacteria. The important fact that isolated specimens of bacteria give rise on solid media to pure colonies, and that by taking advantage of this fact the various species in a mixture may be isolated, escaped the notice of these observers, and it was left to Koch, by his clear demonstration of the utility of this method, to place further investigation on a firm foundation. A pupil of Klebs, Brown (1877), endeavoured to obtain cultures of *Micrococcus diphtheriticus* by breaking a fresh egg under an antiseptic spray and transferring its contents into a previously heated glass vessel plugged with wool, and inoculating the medium with a particle from a swollen lymphatic gland. In this way he obtained a growth of both monads and micrococci which when inoculated into the cornea of a rabbit gave rise to rapid micrococcal infiltration. In transverse sections through the tonsil of a woman exhibiting a firmly adherent greyish yellow deposit he found after staining with haematoxylin three layers, an uppermost layer consisting of fungus rods disposed vertically to the surface, a second layer composed of a coarse fibrinous network in whose meshes dense balls of micrococci were embedded, and finally a third layer consisting of connective tissue densely infiltrated with cells. Klebs, holding Hallier's views, believed that the fungus threads and the micrococci were special developmental forms of the organism to which he had given the name of *Microsporon diphtheriticum*. By means of cultures of this organism, which he considered to be pure, he attempted unsuccessfully to infect animals.

---

[1] Isinglass confined in specially constructed glass capsules (see Löffler, 1887, p. 95).

Several observers gave special attention to the study of the organs of persons who had died from diphtheria. In four cases of diphtheritic myocarditis Rosenbach (1877) found granules and chains of granules, which became brown on the addition of iodine and sulphuric acid, in the connective tissue spaces. He introduced fragments of heart muscle and kidney into the muscles and trachea of the rabbit with remarkable results, namely haemorrhagic inflammation of the muscles in a broad zone round the seat of inoculation, in which could be demonstrated large numbers of spherical and rod-shaped bacteria. Two out of three rabbits, inoculated in the trachea with pieces of heart muscle taken seven to eight hours after death from a child aged 2½ years, who had died of diphtheria in 117 hours, died from symptoms of tracheal obstruction within 24 hours. These inoculations were made by stitching pieces of heart muscle to the rabbits' tracheae, besides introducing scrapings. White, compact, adherent, tubular membranes more than 1 mm. in thickness were found covering the greatly swollen mucous membrane which showed numerous haemorrhages. On the other hand he did not get similar results in 24 inoculations with heart muscle taken at various periods after death, nor in four inoculations with fresh erysipelas membrane. Cultures made in neutral egg-albumen peptone solution with pieces of heart, kidney, and coughed up membrane showed large spherical bacteria and bacilli of three different forms. Rabbits inoculated with a culture from the membrane were not affected. From these results Rosenbach concluded that culture experiments were of little value. Extended investigations were also undertaken by Wood and Formad (1882) during an epidemic which broke out suddenly in a small town. In seven out of 14 cases of diphtheria micrococci were found in the blood. Among seven cases of sporadic diphtheria they were, however, only found in a single case. In these diphtheritic membranes they found micrococci of two forms, small and large, but no bacilli. Numerous experiments on rabbits showed that the material obtained from the cases which occurred during the epidemic was more active than that obtained from sporadic cases. Three rabbits inoculated in the trachea with pieces of membrane died. In one the trachea was filled with exudation which extended into the pharynx; in the second false membrane was found in the larynx only; in the third no such membrane was present. Cultures made with portions of membrane and blood in moist chambers on aqueous humour from rabbits, chicken broth, and ascitic fluid yielded micrococci of various sizes, and in some cases bacteria.

To prevent the growth of *Bacterium termo* these investigators kept their cultures at 40° C., because they found this organism disappeared at that temperature. The cocci grew luxuriantly in the first generation, but after a few generations the growth ceased. Inoculations from various culture generations were made into 21 rabbits. In one instance only, after the inoculation of cocci of the second generation into the thigh, did they find micrococci in the blood. They considered that their failure to find the cocci in the blood in the other cases was due to attenuation. In their opinion the diphtheria micrococcus was a virulent variety of the micrococci constantly present in healthy and inflamed throats. Fürbringer (1883), on the other hand, who examined the kidneys from a large number of cases of diphtheria by the most recent staining methods, found no micro-organisms in these organs, confirming the negative results of Babbe, Weigert (1879), Wagner, etc. In two instances only did he find coccus-like bodies in the kidneys. Both these specimens were derived from children in whom the diphtheritic lesions had been complicated by septic processes. He came to the conclusion therefore, that in the few well established cases in which bacteria had been demonstrated in the organs, their presence was due to secondary complications. At the Congress für innere Medicin, held at Wiesbaden in 1883, Klebs gave an account of his most recent researches, and endeavoured to show that there was a second form of diphtheria besides that set up by the *Microsporon diphtheriticum*. He stated that in sections stained with methylene blue he had found near the surface of the membrane a layer containing extremely short, slender, irregularly disposed rods, apparently embedded in a jelly-like substance. The rods were of uniform length and extremely slender, on the whole barely attaining the dimensions of tubercle bacilli. Spores were frequently present at each end of the rod. When the membranes were dried at room temperature over sulphuric acid the spores increased in numbers, some organisms showing as many as four spores. He stated that he had been unable to obtain pure cultures of the rods. Edlefsen (1883), in the discussion which followed, confirmed the common occurrence of such bacilli in the cases of diphtheria which had come under his observation in Kiel.

In the same year the essay by Heubner, which obtained the prize offered by the Empress Augusta for the investigation of diphtheria, was published. Heubner (1883) attempted to produce an artificial diphtheritic process in the bladder of the rabbit by mechanical means, and then by the inoculation of membranes to superadd a general disease. The animals died from an acute infective disease, with extensive lesions of the bladder, which could be transmitted to other animals by the inoculation of fragments of the mucous membrane of the bladder. The organisms, "micrococci, or perhaps more correctly speaking, short rods, arranged in pairs or in short chains," were found only in the vessels. If these easily demonstrable micrococci or rods were the infective agents in human diphtheria, Heubner thought that it ought to be easy to demonstrate their presence in the vessels of the affected mucous membrane of human beings, in those cases at least in which the disease ended in death. This however he was never able to do, although amongst other specimens he examined serial sections through the uvula, on which deposits had been present a short time previously which contained these bacteria. He came to the conclusion therefore that the bacteria present in the membranes were mere saprophytes, and that the organism of diphtheria had not so far been found.

Koch had shown that his three postulates must be fulfilled before the relation of a micro-organism to a disease could be proved. Firstly, the organisms must always be found arranged in a characteristic manner in the affected part; secondly, these organisms must be isolated and obtained in pure culture; thirdly, it must be possible to reproduce experimentally the disease by the inoculation of pure cultures.

The Discovery of the Diphtheria bacillus by Loeffler.

None of the investigations published up to this time had satisfied these three postulates. Loeffler, however, in 1884, in a paper entitled "Untersuchungen über die Bedeutung der Mikroorganismen für die Entstehung der Diphtherie beim Menschen, bei der Taube und beim Kalbe," satisfied Koch's requirements in regard to diphtheria. In the first place he examined sections of the locally affected parts and also of the organs of patients who had died of diphtheria, stained by a special alkaline solution of methylene blue consisting of 30 c.c. of concentrated alcoholic solution of methylene blue added to 100 c.c. of potassium hydrate solution (1 : 10,000 in water). The material was obtained partly from the Berlin hospitals and partly from Obuchow's Hospital in St Petersburg, and included specimens from cases of scarlatinal diphtheria. These investigations showed that the lesions were not always uniform in character, but could be arranged in various groups. The first group was characterised by loss of substance and the presence of greyish yellow necrotic material, false membranes being absent. All the cases of post-scarlatinal diphtheria belonged to this group. Micrococci, arranged in chains, were found pushing their way in wedge- or tongue-like masses into the tissues and leaving necrotic areas behind them. Masses of micrococci were also found in the organs. In the second group thick false membranes occurred upon the deeply congested mucous membranes of the throat, larynx or bronchi. Masses of bacteria of various kinds were found covering the surface of the false membranes in disorderly confusion, and more deeply small rods arranged in groups and staining most intensely with methylene blue. In the neighbourhood of these rods cells were numerous. The greater part of the false membrane consisted of a fibrinous layer containing but few cells and no bacteria, and lying upon the dilated blood vessels of the mucous membrane. In a small number of cases, showing membranes of this variety, rods were not found. Consequently, if the rods represented the organisms of diphtheria, it was necessary to assume that they had again been eliminated before death took place. Anatomical investigations therefore gave no conclusive proofs of the specific significance of either of the two kinds of bacteria which could usually be demonstrated by staining methods. It was necessary therefore to obtain pure cultures of these two species of bacteria and to discover whether either of them was capable of producing in animals a disease analogous to human diphtheria. Cultivations of the chain-forming micrococci were easily made in peptone gelatin. The cocci obtained in pure cultures grew exceedingly well in broth containing 1 % peptone, ·5 % of salt and ·5 % of grape sugar, in the form of small delicate, curly flocculi which consisted of much interlaced chains sometimes composed of as many as 100 individuals. Abundant growth was also obtained on solidified serum containing 25 % of the above broth. Experiments were made with pure cultures on a large number of animals, mice, field-mice, guinea-pigs, rabbits, dogs, monkeys and birds, partly by feeding, and partly by subcutaneous, intramuscular, intratracheal, corneal and intravenous inoculations. Certain animals, especially mice, died from septicaemia, with numerous chains of cocci in the blood, but in no case was a disease produced which resembled diphtheria. Since these organisms were only discovered in a limited number of cases of human diphtheria, and since they had been found similarly arranged in the organs in other infective diseases which are associated with lesions of the mucous membrane, Loeffler came to the conclusion that in diphtheria also the chain-forming micrococci were merely of secondary importance, occasionally invading the body under favourable conditions and giving rise to local and general complications. Loeffler also regarded it as possible that the chain-forming micrococci might set up a disease resembling diphtheria by attacking the mucous membranes of the throat and multiplying in the lymph-spaces.

Although the rods were undoubtedly present in the membranes, colonies of them failed to develop in a number of culture experiments. Loeffler therefore made use of another nutrient medium, solidified blood-serum containing 25 % of broth, and obtained in the first experiment a positive result. Besides numerous small transparent colonies of cocci there grew upon the surface of the serum distinct, yellowish-white colonies composed of rods which were identical in appearance with those found in sections of membranes.

Further investigations showed that the nutrient medium made use of was in fact the optimum medium for the cultivation of the rods, and that by its help these could be easily isolated from every kind of diphtheritic material in which they were present. With the pure cultures obtained from a number of cases experiments were made on several species of animals. Mice and rats were not affected by inoculations, but guinea-pigs died with characteristic lesions, greyish-white pseudo-membranous masses at the seat of inoculation, haemorrhagic oedema, effusion into the pleural cavities, lobular consolidation of the lungs, catarrhal inflammation of the kidneys and deep reddening of the suprarenal capsules. The bacilli were found usually in small numbers only at the seat of inoculation, but were never observed in the organs. These facts "clearly indicated that a poison produced at the seat of inoculation must have circulated in the blood." Characteristic and striking results were obtained by inoculation into rabbits. After inoculation upon the conjunctiva the parts became much swollen and covered with whitish tough membranes. Inoculations into the opened trachea were followed by the development of intense tracheitis, leading in the majority of cases to the formation of more or less extensive false membranes, which occasionally extended into the bronchi. The operation wounds frequently became covered with fibrinous deposits accompanied by haemorrhagic oedema of the neck. Extension of the process from the trachea into the pharynx was never observed. In the false membranes the bacilli were only found in small numbers, and were only observed in those parts of the mucous membrane which were injured during inoculation and appeared to be totally absent from the internal organs. Since the animals died, in spite of the scarcity of the bacilli, Loeffler considered that death was due to a chemical poison produced by the bacilli at the site of inoculation and absorbed. A monkey, which was but little affected by inoculation on the mucous membrane, developed an extensive oedematous infiltration after subcutaneous inoculation of the bacilli. In small birds intramuscular inoculation was rapidly fatal, and intratracheal inoculation led to the formation of compact, yellow, fibrinous false membranes in fowls and pigeons. Attempts to infect animals by the inhalation of the bacilli or by inoculations on uninjured surfaces were unsuccessful. On the other hand even slight injuries to the mucous surfaces rendered them susceptible to the action of the bacilli. In the female guinea-pig, for example, diphtheritic lesions of the vagina[1] could easily be produced.

Loeffler then proceeded to investigate the question of the occasional occurrence of bacilli in the mouths of healthy individuals. He examined by means of cultures on serum the buccal mucus and the deposits on the teeth of 10 adults and of 20 children from 1—8 years of age. In three cases he found large whitish colonies consisting of short rods which did not exhibit the peculiar forms characteristic of the diphtheria rods, and guinea-pigs inoculated with pure cultures remained well. In a single instance he observed colonies of bacilli identical with the rods found in diphtheria in morphology, cultural characters and action on guinea-pigs. This circumstance was the main reason which prevented Loeffler from unreservedly recognising the bacilli which he had investigated as the causal agents in diphtheria. In addition other reasons compelled him to hesitate. The bacilli, for example, had not been found in all the sections, the false membranes produced by inoculation in tracheotomised rabbits did not show exactly the same arrangement as in the human membranes, and lastly, in those experimental animals which had survived the sequelae characteristic of diphtheria had not been observed. He pointed out, however, that these considerations by no means excluded the possibility that these particular bacilli might be the causal agents of diphtheria. In regard to the discovery of virulent bacilli in the mouth of a healthy child he thought that at a time when diphtheria was common the specific organism might possibly occur in the throat of a child without setting up any symptom of the disease. In any case, since the necessary material could be easily obtained and the conditions for growth were known, it was desirable, if possible, to determine what chemical substances were produced in the growth of bacilli. If the same chemical products could be demonstrated in the culture media, in the bodies of the inoculated guinea-pigs and in the organs of affected persons, a weighty argument in favour of the bacillary nature of diphtheria would be obtained.

Since instances of diphtheria-like diseases in animals had frequently been described which might be due to the same agent which produced human diphtheria and which might therefore be of the greatest importance in spreading the disease, Loeffler studied the diphtheria-like diseases of calves and pigeons. He found that the diphtheria of the calf and of the pigeon were caused by different species of bacteria easily distinguishable from one another, and also from the bacilli of human diphtheria. Consequently these animal diseases could not play any part in the etiology of human diphtheria.

---

[1] The vagina is normally closed by a fold of skin, which can be pulled aside by slight traction.

In regard to the prophylaxis and treatment of diphtheria, Loeffler drew the following conclusions from his investigations: In diphtheria changes first occur in those places on which the virus is deposited, usually the pharyngeal region. Here the multiplication of the bacteria occurs, and the secretions become loaded with them. Individuals suffering from the disease should therefore be isolated, and all materials soiled with the secretions destroyed. Further, since in the act of coughing infective particles might be liberated and then become deposited on the floor and on the walls, the rooms inhabited by the sick persons ought to be disinfected. Lastly as a prophylactic measure all persons suffering from lesions of the mucous membranes ought to be protected from the possibility of infection. In regard to treatment his investigations showed that the affected parts should be treated with disinfectants as early as possible, that the products of the disease ought to be removed, and that remedies ought to be employed to combat the effects of the bacterial poison.

On the 21st April, 1887, Loeffler communicated to a Meeting of the Berlin Army Medical Society some new and important observations which strengthened the arguments in favour of regarding the bacilli, which he had cultivated, as the causal agents of diphtheria. He communicated the results of an autopsy made by Professor Heller of Kiel immediately after death, in which typical diphtheria deposits were found in the stomach. In this instance numerous bacteria were found on the surface of the mucous membrane of the stomach, but the characteristic bacilli were only observed in the parts affected by diphtheria. He also cited the results of cultural investigations on 10 cases of diphtheria within 24 hours of the appearance of symptoms. In all these cases the typical bacilli were found. From one of these cases he isolated, together with virulent diphtheria bacilli, organisms which were morphologically similar to the diphtheria bacilli, but somewhat smaller; these, however, when inoculated into guinea-pigs were found to be completely non-virulent. He pointed out the importance of the fact that there exist non-virulent organisms closely resembling diphtheria bacilli, and urged the necessity for performing in all cases inoculations on guinea-pigs, animals which had been proved by numerous experiments to be peculiarly suitable for the purpose. He further pointed out that other bacilli belonging to the same group might be found as in the case of cholera, but that careful consideration of all the morphological and biological peculiarities would, no doubt, render a distinction between true and pseudo-diphtheria bacilli possible.

Several years passed before Loeffler's discoveries received attention from other observers. Babes, in 1886, was the first to seriously attack the problem. He was followed by Sörensen (1886), Wyssokowitsch (1886), D'Espine (1886–8), von Hofmann-Wellenhof (1888), Roux and Yersin (1888), Ortmann (1889), Zarniko (1889), Marignac and D'Espine (1890), Kolisko and Paltauf (1889), Spronck, Wintgens and van den Brink (1889), all of whom demonstrated the almost constant occurrence of the characteristic bacilli in typical cases of epidemic diphtheria. The only observers who failed to find the bacilli were Prudden (1889), who, like Wood and Formad at an earlier period, constantly found streptococci in diphtheritic lesions, and Emmerich who, at the Congress of Hygiene held at the Hague in 1884, stated that he had found plump short rods which grew luxuriantly upon bouillon-peptone-gelatin and potato and produced croupous membranes on the mucous membrane of the trachea of the rabbit. These he considered to be the exciting cause of diphtheria. In 1890 Loeffler (1890) reviewed the investigations that had been made up to that time and published the results of further investigations, which had already (1888) been communicated to the Greifswald Medical Society. The objections advanced against the etiological significance of the diphtheria bacillus he was now able to overthrow point by point.

He pointed out in a short review that his numerous positive experiments gave abundant proof of the constant association of the bacilli he had cultivated with diphtheritic diseases, and that evidence was lacking that they were "common if not regular inhabitants of the pharynx," as Baumgarten had maintained. The occurrence of organisms resembling diphtheria bacilli both in diphtheria itself, in other diseased conditions and on healthy mucous membranes, was discussed at length, and the opinion expressed that the bacilli described in his first communication, the non-pathogenic organisms cultivated from numerous cases by von Hofmann-Wellenhof on meat-peptone-agar at ordinary room temperature, the organisms found in xerosis by Kutschbert and

E. Neisser (1883) and Ernst (1888 and 1889), and by Babes in three cases of trachoma, and the diphtheria-like bacillus of Zarniko, were various species belonging to the same natural group as the diphtheria bacillus. He stated that they could readily be distinguished from the diphtheria bacillus by certain morphological differences, the absence of pathogenicity for the guinea-pig, and the luxuriant growth on ordinary agar at room temperature. He admitted that the classification of certain bacilli found in non-diphtheritic processes, especially measles and scarlatina, which were indistinguishable in form or virulence from the bacilli found in diphtheria, was a difficult matter. In these cases he thought we might be dealing with the complication of these diseases with true diphtheria. In this group he placed the bacillus found by himself in the throat of a healthy child, and another found by Hofmann-Wellenhof under similar circumstances, and also the bacilli cultivated by Babes from three cases of follicular tonsilitis in adults, from the margin of a perforating ulcer of the palate in a case of hereditary syphilis, from a case of genuine lobar croupous pneumonia and from a case of stomatitis gangrenosa. He drew attention to the facts that neither the production of false membranes upon the conjunctival or tracheal mucous membrane nor the death of inoculated guinea-pigs were absolutely pathognomic criteria of true diphtheria bacilli, since bacilli existed which killed guinea-pigs with the formation of haemorrhagic oedema, as well as bacilli which produced false membranes on the mucous surfaces. The bacilli cultivated by Babes from two cases of diphtheria in human beings were cited as examples of the latter. They could easily be distinguished from diphtheria bacilli by the formation of distinct, oval spores resisting a temperature of 100° C.

Loeffler did not consider the occurrence of bacilli, indistinguishable from the true diphtheria bacilli, in non-diphtheritic lesions or upon healthy mucous membranes an argument against the specific nature of the bacilli constantly found in diphtheria, because diphtheria is everywhere endemic, and therefore its causal agent might be deposited on mucous surfaces without giving rise to the characteristic morbid phenomena.

Loeffler also communicated the important fact that two guinea-pigs, amongst the few which survived an inoculation with diphtheria bacilli, suffered from extensive necrosis of the skin, followed by very marked paralytic phenomena which only slowly subsided. After surviving this paralysis the animals were capable of withstanding repeated inoculations with cultures of bacilli without considerable local reaction.

He also laid stress on the fact that he had succeeded, in the years

1887 and 1888, in obtaining the poison which he had postulated to be the disease-producing agent of the diphtheria bacillus, giving the following description of his methods:

> "I have pointed out that one of the most striking phenomena in inoculation experiments on guinea-pigs with the bacilli is the circumstance that, in spite of the severity of the lesions in the kidneys and pleural cavities, bacilli were never found in the organs of the inoculated animals. I concluded from these facts that the bacilli produced a poison which acted injuriously on the blood vessels, and naturally sought to obtain this poison if possible from cultures and to gain some idea of its nature. A year ago I described the results of the attempts I had made in the autumn of 1887 and summer of 1888, but in the interests of those who were not present at that meeting, I briefly repeat my procedures:
>
> At the end of October 1887 I sowed a number of flasks containing 90 grammes ox-bouillon (1 °/₀ peptone, 1 °/₀ grape sugar and 0·5 °/₀ salt, neutralised with phosphate of potash) with pure cultures of the bacilli. At the end of three days deposits were seen on the walls of the flasks, and the reaction in all cases had become acid. One flask was shaken up with ether for several days, and another was evaporated down to 10 c.c. on a water-bath. Four syringe-fulls of ether shaken with the bouillon were injected into a guinea-pig, 6 into a rabbit and half a syringe-full into a mouse. Apart from the transient action of the ether no symptoms of illness were manifested. The bouillon, which had been reduced by evaporation to 10 c.c., was also found to be quite innocuous. Other matters prevented me from continuing these investigations until the summer of 1888. I then decided to adopt another method of obtaining the poison.
>
> The bacterial poison resembled in its action the poison obtained from Jequiriti seeds, which causes inflammation and the production of false membranes when placed upon the mucous membranes either of men or animals. Salomonsen and Christmas-Dirckinck-Holmfeld had shown that the Jequiriti poison was a kind of enzyme, soluble in water and glycerine, insoluble in alcohol, ether, benzine and chloroform and completely inactivated by an exposure to 65—70° C. for one hour. Earlier experiments had convinced me that cultures heated for half an hour at 60° C. were always sterile, and when injected subcutaneously produced no effect on animals. Thinking that the bacillary poison might also be a kind of enzyme, I carefully followed the method of examination used by the Danish investigators, and, in conjunction with my assistant, Mr Holz, made a very large number of experiments. Fresh meat minced and exactly neutralised was placed in flasks in quantities of 200—500 grms., and sterilised. After inoculation with pure cultures of the bacilli the flasks were incubated at 37° C. The cultures were examined four or five days after inoculation, and, if pure, glycerin was added and the flask was vigorously shaken. After a further period of 24 hours incubation the glycerin extract was precipitated by the addition of 5 times its volume of absolute alcohol. After standing for 24 hours the voluminous flocculent precipitate was separated by filtration, washed with alcohol, carefully dried and dissolved in a very small quantity of water. This solution was again mixed with 5 times its volume of absolute alcohol, and at the same time carbonic acid gas was passed through the fluid. The white precipitate was again obtained by filtration, dried and finally dissolved in water. With this

> solution guinea-pigs were inoculated. Solutions containing 0·1—0·2 grm. of the substance produced violent pains in guinea-pigs after subcutaneous injection. The animals bristled up their hair and raised themselves up on their feet, uttering loud cries. Compact fibrinous nodules and haemorrhages in the muscles and oedema in the neighbourhood developed during the next two days at the seat of injection. The lymphatic glands draining the inoculated region were swollen and haemorrhagic, and the organs, especially the kidneys, strongly hyperaemic. The cutaneous lesions ended in necrosis of the skin. A pregnant animal aborted. Injections into the peritoneal cavity killed the animals with all the phenomena of a severe inflammation of the peritoneum. The local phenomena were consequently the same as after the injection of the bacilli. Control experiments with uninoculated meat treated in the same way produced no effects. The experiment was repeated five times with the same result. The poison was consequently a kind of enzyme, similar to that obtained from Jequiriti seeds, but the action of the latter was more intense, for, according to the statements of the Danish investigators, the glycerine extract from one hundred thousandth of a gramme of the Jequiriti seed set up a distinct inflammation of the conjunctiva."

The French investigators Roux and Yersin (1888), using entirely different methods, reached exactly the same conclusions as to the nature of the poison manufactured by the bacilli. In the first place, they filtered seven days old cultures made in veal bouillon through porcelain filters, and injected the filtrate freed from the bacilli into guinea-pigs. Following the injection of enormous doses, 35 c.c., the animals became ill after 2—3 days, the hair bristling up, and the urine showing traces of blood. Death took place in 5 or 6 days, the later symptoms being irregular respiration, and great weakness of the hind legs. Autopsies showed hyperaemia of the kidneys, ecchymoses along the vessels and serous exudations into the pleura. Cultures six weeks old gave a much more poisonous filtrate, and its effects appeared earlier. Even within a few hours diarrhoea set in. Older cultures produced a still more poisonous filtrate, for example, ⅛th of a c.c. of a 30 days old culture was sufficient to kill a guinea-pig. The filtrate also produced an effect on rabbits, sheep, dogs and birds. In many cases where the dogs did not die they exhibited paralysis. Rats and mice were found to be as resistant to the action of the poison as they are to injections of the bacilli. Exposure to a temperature of 58° C. for two hours greatly diminished the power of the poison, and an exposure to 100° C. for twenty minutes completely destroyed it. The poison which had been attenuated by heat set up chronic phenomena, the animals becoming emaciated, paralysed in the hind limbs and eventually dying. Exposure to sunlight and air diminished the activity of the poison. Roux and Yersin were able to produce these phenomena with the filtered urine of individuals suffering from diphtheria, or with extracts obtained from the organs of persons who had died of diphtheria.

By evaporation *in vacuo* and extraction with alcohol a non-poisonous extract was obtained. The poison was consequently insoluble in alcohol, and in fact could be precipitated by alcohol. From old cultures of the bacilli, which had lost their acid reaction and become alkaline, they were able to obtain the poison mechanically by fractional precipitation with calcium chloride. The toxicity of this precipitate was very great. According to Roux and Yersin $\frac{4}{10}$ of a mgrm. of this substance was sufficient to kill eight guinea-pigs or two rabbits. The poison, contained in the calcium chloride precipitate, could be heated in the dry state to 70° C. and even to 100° C. for twenty minutes without losing its activity. The properties of the poison led Roux and Yersin to the conclusion that it was a kind of enzyme. They came therefore to the same conclusions as Loeffler, but by another line of investigation.

The experiments of Roux and Yersin explain the reason why Loeffler obtained a comparatively weak poison by extraction with glycerine. The latter worked with young cultures 4—5 days old, not realising that on artificial media the bulk of the poison is formed later. Loeffler made use of cultures a few days old because such cultures always manifested the greatest action when injected into guinea-pigs, whilst older cultures 4—5 weeks old were often more uncertain in their action, probably owing to the fact that many of the organisms were dead. Kolisko and Paltauf (1889) independently made use of the same methods as Roux and Yersin, and succeeded in separating the diphtheria poison from the bacilli. They filtered 14 days old living broth cultures through Breyer's micro-membrane-filter and injected the germ-free filtrate subcutaneously into guinea-pigs in doses of 1 c.c. They observed that nodular infiltration occurred at the seat of injection leading to necrosis and the formation of ulcers, *i.e.* the same phenomena which Loeffler had observed after the injection of the substance precipitated by alcohol from the glycerine extract. It must further be pointed out that although the poison obtained by Roux and Yersin acted with such extraordinary virulence when injected subcutaneously into guinea-pigs and pigeons, it could be swallowed without danger.

Now began a new era in the investigation of diphtheria.

Everywhere the bacteriological study of diphtheria and of the diseases resembling it was prosecuted with vigour; Brieger and Fränkel (1890), Beck (1890), A. Baginsky (1892), and H. Kossel (1895) in Berlin, Escherich (1890) in Munich, Klein (1890) in London, Tangl

(1890) in Tübingen, Welch and Abbott (1891), Prudden (1891) and Park (1892) in America, Lefèvre (1891), Roux and Yersin (1890), Morel (1891) and Martin (1892) in Paris, and many other investigators, examined large numbers of cases. The first result of these comprehensive investigations was the discovery of the fact that the typical virulent diphtheria bacillus was present in only 60—80% of the cases investigated. It became apparent therefore, after allowing for a certain proportion of negative results owing to inadequate technique, that a certain percentage of cases, clinically diagnosed as diphtheria, were not true examples of that disease. These investigations also demonstrated that the form of the bacillus varied within certain limits, so that a short and a long bacillus had to be distinguished. It was further shown that the virulence of typical diphtheria bacilli found in the different cases was extremely variable; in fact in some cases highly virulent, while in others attenuated, and even completely non-virulent diphtheria bacilli might be found. A distinction had consequently to be made between virulent and non-virulent true diphtheria bacilli on the one hand and pseudo-diphtheria bacilli on the other. The differentiation of the virulent diphtheria bacilli from the pseudo-diphtheria bacilli required a prolonged investigation, including the making of pure cultures and the inoculation of animals. A rapid and reliable means of diagnosis was still lacking.

Max Neisser (1897) discovered a special method of staining[1], capable of being used for differential diagnosis, which consisted in treating preparations from young cultures on Loeffler's serum for 1—3 seconds with a 1:1,000 solution of methylene blue containing 5% of acetic acid, and subsequently stained for 3—5 seconds in 2:1,000 solution of Bismarck brown. The body of the diphtheria bacillus is stained brown and contains blue granules, usually situated at the ends, but sometimes also in the middle. In pseudo-diphtheria bacilli blue granules are not found. The granule staining has on the whole stood the test of extensive subsequent use. In certain cases however it has failed. Lubowsky (1900) later devised another method, which consisted in immunising animals with the non-virulent bacilli and testing the agglutinating properties of their serum on true and pseudo-diphtheria bacilli. The true diphtheria bacilli are agglutinated, but not the pseudo-diphtheria bacilli. The agglutination test however, owing to the difficulty of preparing uniform, homogeneous suspensions of isolated bacilli, cannot be applied except with great difficulty. It was only by extracting the bodies of the bacilli with 1% solution of ethylenediamin, according to Aronson's method, and the injection of these extracts into rabbits, that Wassermann (1902) succeeded in preparing a serviceable material suitable for the differential testing by agglutination and precipitation, so that the differentiation of the true diphtheria bacillus from other similar bacilli belonging to the same natural family can now be effected with ease.

---

[1] For recent modifications see Chapter IV.

The extensive cultural investigations of diphtheria led to the recognition of the important influence exerted by secondary bacteria on the course of the disease. Escherich, Zarniko, Beck, Roux, Barbier (1891), Martin, Prudden, Prudden and Northrup (1889), Schweighofer (1892), Troje (1892), Koplick (1892), Goldscheider (1893) and others demonstrated that other pathogenic bacteria are almost invariably present on serum cultures together with diphtheria bacilli. Streptococci are the most frequent associates. Pyogenic staphylococci and pneumococci are less common. Experimental investigations upon the combined action of the diphtheria bacilli and the above mentioned pathogenic organisms showed that the simultaneous injection of streptococci could increase the virulence of the diphtheria bacilli. Roux and Yersin tried to raise the virulence of slightly virulent races of bacilli by this means.

As a rule it was found that the bacilli remained localised at the seat of invasion and multiplied there, manufacturing their poison. In this respect the experiments on guinea-pigs and the observations on human subjects were in complete agreement. In certain cases, however, a more general dissemination of the bacilli was noticed.

Loeffler in his first publication had recorded the presence of the bacilli in the epithelium of the bronchi and in contents of the alveoli and the perivascular lymph-spaces. Babes, Escherich, Kolisko and Paltauf, and Behring and Wernicke (1892) had also in certain cases observed the bacilli in internal organs.

Frosch (1893) was able to demonstrate the presence of the bacilli in the brain, lungs, liver, spleen, kidneys, cervical and bronchial glands, heart's blood, pericardial and pleural fluids in 10 out of 14 cases. R. Pfeiffer (1888) also found them in the heart's blood, and Kutscher (1894) demonstrated them in 8 out of 9 stained preparations from the lungs and in one from the kidney. Nowack (1896) proved the presence of diphtheria bacilli in the blood in 9 out of 22 cases by means of cultures. In the majority of his cases, however, he also found streptococci, to which he attributed the severity of the symptoms.

Important observations were also made on the duration of life of the diphtheria bacillus outside the body, and the conditions which affect it.

Loeffler (1890) found that bacilli from cultures dried on silk threads and kept under ordinary conditions remained alive for 3 to 4 weeks, while in the desiccator they might remain alive up to 10 or 14 weeks. From small dried pieces of membrane he was able to obtain living bacilli in large numbers at the end of 4 weeks, and in some cases even at the end of 9 weeks. From thicker pieces numerous colonies were obtained after 9 to 10 weeks, and isolated colonies after 13 or 14 weeks. After 16 weeks the bacilli could never be cultivated.

From pieces of membrane dried on linen and kept in the dark at room temperature, Roux and Yersin (1890) were able to cultivate numerous colonies at the end of 3 to 4 months. Abel (1893) was able to demonstrate living bacilli on a child's toy 6 months after infection.

The experiments of Abel (1893) and Kasansky (1899) proved that the bacilli are very resistant to cold, but the observations of Gehrke (1896) and others showed that they are greatly affected by light.

Several observers showed that the bacilli were capable of remaining alive for prolonged periods in culture. Loeffler, for example, found cultures to be still alive up to 331 days, Abel up to 373 days, Klein (1890) up to the end of 18 months, and Kasansky (1899) up to 527 days. How long diphtheria bacilli retain their vitality on the mucous membranes of affected individuals was a question which required careful experiment. Escherich (1890) stated that he was still able to demonstrate bacilli 1 to 3 days after the deposits had disappeared, but Roux and Yersin (1890) later found the bacilli 2 weeks after the disappearance of the membrane. Loeffler read to the International Medical Congress, held at Berlin in 1890, a report upon a case in which virulent bacilli could be demonstrated for nearly 3 weeks after the temperature had again become normal. Still later Abel (1894) in one instance found the bacilli 65 days after the disappearance of the membrane. Numerous observers have more recently confirmed and extended these observations (see Index).

In consequence of these facts Loeffler (1894), as President of the German Diphtheria Committee, proposed to the 8th International Congress for Hygiene and Demography held in Budapest that "Convalescents from diphtheria should not be permitted to resume normal social intercourse until the complete disappearance of the bacilli had been demonstrated by bacteriological examination."

The occurrence of virulent bacilli, capable of conveying infection, on the mucous membranes of convalescents long after recovery from the disease explained the important observation which Loeffler had noted in his first communication, and which prevented the acceptance of the diphtheria bacillus as the causal factor for a long time, namely the occurrence of diphtheria bacilli in the throats of healthy individuals. Numerous recent observations have proved the frequency of this occurrence (see Index), and similar conditions have been proved to prevail in cholera, in which disease only a portion of the individuals who acquire the organisms become ill.

One of the first of the series of experiments on a large scale, which proved the frequency with which healthy individuals became infected, was made by Loeffler during an epidemic which raged in Greifswald. In recording the outbreak he says:

> "After cases of diphtheria had occurred in several schools, the Health Commission ordered a medical examination of the whole of the school children, which was to be repeated from time to time and to be carried out by the various medical departments of the University. The Institute of Hygiene participated in these investigations. I took advantage of this favourable opportunity and, assisted by Dr Abel, made a bacteriological examination of 160 children. True diphtheria bacilli were found in cultures from four of these. Personal enquiry was at once made in the school and elicited the fact that one of the children, who was harbouring the bacilli and who had not shown any signs of illness, was absent from school. The child had developed diphtheria. The second child was found to be suffering from mild diphtheria. This child was somewhat torpid, but had made no complaints at home and now sat amongst its companions suffering from diphtheria. The third child had slight inflammation of the tonsils, but was otherwise well, and the fourth child was quite well. The two latter did not become ill, although the bacilli did not disappear from their throats for three and ten days respectively. In 11 of the children we found in addition the so-called pseudo-diphtheria bacilli, which are regarded by many as attenuated or, more correctly, non-virulent forms of the diphtheria bacillus, but which I look upon as quite a different species of bacterium."

In a similar manner Fibiger (1897) succeeded in checking an outbreak which had broken out on more than one occasion (see Index).

A great number of the healthy individuals in whom the bacilli were found had come into close contact with persons attacked by diphtheria, and the possibility that the disease might be spread by such healthy bearers of the bacilli was obvious. Subsequent investigations have proved that this is frequently the case. The reason why bearers of the virulent bacilli do not fall ill was first discovered by Abel (1894) and later, but independently, by Wassermann (1895). Loeffler reported upon this as follows:

> "Dr Abel has found that the blood of many healthy individuals, who have not recently suffered from diphtheria, possesses the property of protecting the guinea-pig against fatal doses of diphtheria bacilli or diphtheria poison. We must assume that the bacilli are unable to injure such immune individuals because the bacillary poison has no effect upon them."

Wassermann, and after him Fischl and Wunschheim (1895) and Schmid and Pflanz (1896), came to the same conclusion. Klemensiewicz and Escherich (1893) had previously shown that protective substances could be demonstrated in the blood of persons who had suffered from diphtheria, and this observation was confirmed by Abel, Orlowsky (1895), Loos (1896) and others.

The fact that sick persons harbouring diphtheria bacilli expel them when speaking, and more especially when coughing, was proved by their occurrence in cultures taken from objects in the immediate vicinity of the sick persons. W. H. Park (1892) found the bacilli on pillows and bed-clothes, and Wright and Emerson (1894) on a brush which had been used to sweep the floor of a diphtheria ward, in the dust clinging to the shoes of a nurse and on the hair of a nurse at the place usually touched when smoothing back the hair with the fingers. Forbes (1895) during an epidemic of diphtheria at Rochester, apparently due to the use of a drinking vessel in common, found the bacilli on the edges of the vessel, and Weichard (1900) once succeeded in demonstrating the bacilli on the glass portions of a sucking-bottle which had recently been used by a sick child, on the handkerchief of a child with a violent cough and on the carpet close to the bed of a sick child.

In view of these positive findings the negative results obtained from the investigations made on this point by Hagemann and Kober (1899) lose their weight.

Observations of this kind proved the necessity for strictly observing the rule which Loeffler had previously laid down, that "In every case of diphtheria it is necessary to carry out disinfection, which should include all objects used by the sick person, and also the patient and the sick room."

No special methods of disinfection are necessary since many observers, amongst them Loeffler (1891), Behring (1894), Boer (1891), D'Espine and Marignac (1890), Aronson (1890), Laser (1894), Dräer (1894), Beck (1901), have proved the relatively small power of resistance possessed by the non-spore-forming diphtheria bacillus against many disinfecting agents.

At an early period in the bacteriological investigations of diphtheria Loeffler (1890) came to the conclusion that under suitable conditions the diphtheria bacillus was capable of multiplying outside the body, because the minimum temperature at which the bacillus grows is about 20° C., a temperature which in summer, and in heated rooms in winter, is very often reached and even far exceeded. Moreover suitable conditions for its growth are not wanting, and Loeffler especially referred to milk in which the bacilli grow well. Outbreaks of diphtheria traceable to the milk traffic have been repeatedly mentioned in literature (see Index).

As early as 1885 Power described a series of epidemics in which he found the distribution of scarlet fever and also of diphtheria to be strictly limited to the customers of one, or a few, milk sellers; and Loeffler, in his address on the hygiene of dairy products to the meeting of the Deutscher Verein für öffentliche Gesundheitspflege, held in Rostock in 1901, mentioned two epidemics described by Deneke at Stendrup and Flensburg, in which only the customers of a certain dairy were attacked.

In Stendrup a woman used to milk her cows immediately after attending on her child which was suffering from diphtheria, and sent out the milk in vessels which she had cleaned.

In Flensburg the two children of the man who brought the milk from the farms and took it to the dairy were suffering from diphtheria. After collecting the milk this man first went home, carried the cans inside and took out his own share of milk. In both these cases every opportunity was afforded for the milk to become infected.

The part played by animal epidemics in the diffusion of human diphtheria has often been studied. Loeffler's observations on the causal agent of pigeon diphtheria, which have already been quoted, were confirmed by Pütz (1887), Cornil and Babes (1890), Méguin (1891) and Ménard (1890). Emmerich discovered both in human diphtheria and in six cases of pigeon diphtheria, bacilli which differed entirely from the diphtheria bacillus. Concetti (1894) succeeded in cultivating from the grey white membrane upon the posterior wall of the throat of pigeons a short bacillus which liquefied gelatine. By means of pure cultures he produced diphtheritic deposits on the mucous membranes of pigeons. The birds died in 6 to 8 days. L. Pfeiffer (1888) found in the exudations from pigeons suffering from diphtheria enormous numbers of flagellata (Protozoa), which he considered to be the cause of the disease. Babes and Puscariu (1890) also found these flagellata, but found together with them the bacillus of pigeon diphtheria corresponding in its cultural characters and pathogenic action with the organism described by Loeffler. On the other hand certain clinical evidence points to some connection between human and avian diphtheria. Gerhardt (1883) stated that during an epidemic, which broke out amongst the fowls in a poultry rearing establishment in Nesselhausen, two-thirds of the day-workers suffered from a diphtheria-like disease, and that a number of them communicated the disease to their children. At that time true diphtheria was not prevalent in the locality. Roth (1883) declared that all hens which ate the false membranes of children suffering from diphtheria fell ill of diphtheria, and Turner (1887) stated that on several occasions an attack of croup in children was preceded by diphtheria amongst the fowls.

Barbier (1899) mentioned the case of a woman 67 years old, who developed all the symptoms of severe diphtheria three days after cleaning out an hen-house occupied by Italian hens which were suffering from diphtheria. No other case existed in the neighbourhood, and she had never been out of the house for the previous three weeks.

Debrie (1892) described an outbreak amongst hens, which were looked after by an attendant attached to the hospital at Sebdou, shortly after the admission of several soldiers suffering from diphtheria. Finally Cole (1894) and Schrevens (1894) described cases of the transmission of fowl diphtheria to children. In none of these cases, however, was there any mention of bacteriological examinations. Loir and Duclaux (1894) had the opportunity of studying a widespread epidemic of avian diphtheria in Tunis, in which frequently two-thirds, and occasionally even the whole, of the poultry died. The cause of this disease was found to be a motile bacillus with blunt ends, which could be found in all the tissues and fluids of fowls which had died from the disease. Cultures of this bacillus produced a luxuriant growth consisting of yellowish-white colonies on potato at ordinary temperature. It did not stain by Gram's method. The organism was pathogenic for the fowl, pigeon, duck, sparrow and rabbit, but not for the guinea-pig, and was therefore entirely different from the bacillus of human diphtheria. Nevertheless it was capable of producing in man a diphtheria-like disease, in which it could be demonstrated in the false membranes. Gallez Jr. (1896) on the other hand considered that it was important to distinguish between the so-called diphtheria of the fowl and a disease known amongst veterinary men as "catarrhe contagieux." He says that in this form of catarrh a bacillus is found only differing from Loeffler's diphtheria bacillus by its lesser degree of virulence. When the virulence of this bacillus is augmented and it is injected in large doses into rabbits it rapidly kills the animals, with lesions similar to those produced by the human diphtheria bacillus. This observer concluded therefore that "catarrhe contagieux" was a common cause of sudden outbreaks of true human diphtheria, an assumption which has up to the present not been confirmed. The literature of this subject has been reviewed by Galli-Valerio (1897).

Loeffler's investigations on the diphtheria-like disease in calves, first described by Dammann (1891), was completely confirmed by Bang (1893). Klein (29. IV. 1890) however has described naturally acquired diphtheria in cats and experimental diphtheria in cows (see Index). Other observers have not confirmed his statements.

Up to the present therefore both experiments and observations indicate that diphtheria is almost entirely spread by human agency.

Numberless investigations on diphtheria bacilli in diphtheritic secretions and in cultures have increased our knowledge of the morphology of the bacillus.

In the year 1890 Klein (1899) observed that the bacilli, found in the infiltrated areas produced in cows by the subcutaneous injection of diphtheria bacilli, had often grown out into threads of great length. These threads were provided with lateral or terminal buds or swellings. He therefore compared the diphtheria bacillus to a mycelial fungus. Kanthack (1896) also observed club-shaped and branched forms. General attention, however, was only drawn to the branching of the diphtheria bacillus by the communications of Babes (1889) and C. Fränkel (1895) on the occurrence of such forms in diphtheria cultures. Bernheim and Folger (1896) later observed branching forms in diphtheritic membranes. Meyerhof (1898) on this account regarded the diphtheria bacillus as a species closely allied to the streptothrices, while Lehmann and Neumann (1896) separated them from the bacteria, and grouped them with the Hyphomycetes under the genus Corynebacterium (*κορύνη*, club).

The Toxin of B. diphtheriae.

The capacity for producing a soluble poison, the most interesting and important property of the diphtheria bacillus, has been closely studied by many investigators. Brieger and Fränkel (1890), like Roux and Yersin, showed that the toxin producing power could be increased or diminished. They found that after the bacilli had been grown for some time on glycerine agar their power of manufacturing poison diminished. They further found that the poison was injured by exposure to 60° C., but not by exposure to 50° C. They finally endeavoured to obtain the poison in a pure form by precipitation with ammonium and sodium sulphate. By repeated precipitation with alcohol and dialysis they obtained a substance which killed animals, in small doses, 2·5 mgrm. per kilo. Owing to the fact that this substance gave the characteristic reactions for albumen they gave to it the name of *toxalbumin.* By evaporation *in vacuo,* dialysis and treatment with 60—70 % alcohol, and simultaneous acidification with acetic acid, Proskauer and Wassermann (1892) obtained a fine white powder oı great toxicity. A similar albumen-like preparation was obtained by extraction with glycerine from the organs of animals and also of human beings who had died from diphtheria, by Brieger and Wassermann (1892). Sidney Martin (1892) obtained similar results. According to this observer there is present in the false membranes a ferment-like body differing from the toxic albumoses.

Gamaleïa (1892) considered the diphtheria poison to be an albuminous body, belonging to the group of *nucleoalbumins.* He believed that the toxin was contained in the bodies of the bacteria and was gradually extracted from them by maceration, because, notwithstanding vigorous growth, the broth at first possesses no toxic properties, whilst the toxicity increases with the age of the culture, which becomes alkaline. Behring (1893) by reflection and experiment arrived at a different view as to the nature of the diphtheria poison. In his opinion the diphtheria poison could not be an enzyme or ferment, because it has not the almost unlimited capacity for action possessed by these bodies.

In the case of the diphtheria poison on the other hand we can calculate with the greatest nicety the amount of the poison that is necessary in order to kill a guinea-pig, rabbit or sheep of known weight and age, or the quantity sufficient to make it ill. Behring thought that the nature of the diphtheria poison was not yet understood, and that observations of Brieger and Fränkel and of Gamaleïa had not materially advanced our knowledge. H. Kossel (1896) thought that the toxin mainly originated in the bodies of the bacteria and was a metabolic product of the diphtheria bacilli. Guinochet proved that it could not be an albuminous body, because the filtrate from a culture grown in urine, free from albumen, was distinctly toxic. Further evidence in this direction was obtained by the experiments of Brieger and Boer (1895). Continuing the work of Knorr (1899), who had shown that the diphtheria poison could be obtained from the culture fluid in a very concentrated form by precipitation with ammonium sulphate, they found that it could be precipitated by zinc chloride and could be again set free by means of sodium phosphate. The product so obtained exhibited neither albumen nor peptone reactions and was found to be optically inactive. They succeeded in isolating at the same time from the bodies of the bacteria another poison which possessed the property of causing necrosis.

Carrying their investigations further they at last succeeded in obtaining the pure toxin by means of a double zinc combination. The body thus obtained they were unable to refer to any definite group of organic substances by the use of the ordinary reagents.

Then came the fundamental researches of Behring and of Ehrlich on the estimation of the strength of the diphtheria poison, and the most important investigations of Ehrlich (1896–98) on the changes taking place in solutions of the diphtheria poison with keeping, and the investigations of Madsen (1897), Arrhenius and Madsen (1902) and Dreyer and Madsen (1902) on the constitution and mode of action of the diphtheria poison. Many investigations were made as to the most suitable media and cultural conditions for the formation of the poison. Especial mention must be made of the paper by Roux on the necessity for an abundant supply of oxygen and the cultivation of the bacilli on the surface of peptonised ox-bouillon, and the demonstration by Spronck (1895) of the influence of the sugar contained in the meat from which the bouillon was made.

Local Treatment of Diphtheria by Chemical Agents.

The most important results however were obtained from experiments which had in view the destruction of the bacilli in the infected areas and the neutralisation of the intoxication caused by the bacillary poison. With regard to local treatment, Loeffler (1891) endeavoured to find means on the one hand to prevent the lodgement of diphtheria bacilli on the mucous membranes, and on the other to destroy the bacilli which had already developed in dense masses on the superficial layers of the false membranes. Experiments on serum cultures showed that corrosive sublimate (1 : 10,000—1 : 15,000), cyanide of mercury (1 : 8,000—1 : 10,000), chloroform water, chlorine water (1 part of chlorine in 1,100 parts of water), or thymol (1 part in 500 parts of 20 % alcohol) acting for 10 seconds prevented the development of the bacteria. The same result was obtained by the continuous action of the vapour of benzol, toluol, several ethers of the aromatic series, as phenetol and anisol, and also several ethereal oils, as the oil of orange-peel, lemon oil and eucalyptus oil. Cultures of diphtheria bacilli were killed in 20 seconds by corrosive sublimate (1 : 1,000), carbolic acid (3 % in 30 % alcohol or 5 % in aqueous solution), bromine (2 % in aqueous solution) and chlorine (1 % aqueous solution).

As, however, 20 seconds proved in practice to be too long, Loeffler tried to find a quicker method of killing the bacilli, and discovered that a mixture consisting of 36 c.c. of toluol, 60 c.c. of absolute alcohol, 4 c.c. of liquor ferri sesquichlorati and 10 grms. of menthol (to hinder pain) acted rapidly and successfully. During an epidemic 71 patients were treated with this mixture and not one of them died, whilst among the other notified cases there was a mortality of 18·2 %. Behring (1890), in collaboration with Boer (1891), observed the effect of various substances on animals infected with diphtheria, and came to the conclusion that only a few of these were of any value as local applications. These were zinc chloride, sodium auric chloride, and iodine trichloride, which, as Behring (1893) pointed out, are closely related to hydrochloric acid. Bretonneau used the latter with success in the treatment of diphtheria of the skin and of the mucous membrane, when the areas of infection were limited; and it had been recommended almost 100 years previous to Bretonneau's time by Gerard van Swieten as an excellent curative remedy. Preparations of mercury, aniline dyes, arsenic, and most of the preparations of the benzol series, as well as many other remedies frequently used in the treatment of diphtheria, were found to be of little value in the treatment of experimental diphtheria, or at any rate very inferior to hydrochloric acid or substances which give off hydrochloric acid or free chlorine in contact with living tissues.

The Discovery of Antitoxins and their Application in the Treatment of Diphtheria by Behring, Roux and others.

The therapeutic investigations of Behring led up to a subject of great importance, the artificial immunisation of animals against diphtheria.

Local treatment was naturally only practicable in cases in which the pharyngeal mucous membrane alone was involved, while in cases of nasal, laryngeal, and bronchial diphtheria little could be effected by local treatment. Moreover, local treatment could not destroy the poison which had already penetrated into the body. As Loeffler had pointed out in his first communication, the principal danger in diphtheria lies in the action of the poison, and the main object of treatment should be its neutralisation. To Behring belongs the distinction of being the first to achieve this object.

Loeffler (1890), in a paper entitled "Der gegenwärtige Stand der Frage nach der Entstehung der Diphtherie," quoted a case in which a guinea-pig recovered from the effect of an injection of diphtheria bacilli after extensive necrosis of the skin. A second injection only produced a slight local swelling, but the animal developed severe paralysis from which it recovered after three weeks. Subsequent injections only produced slight local reactions, showing that the animal had become immune, and the experiment therefore showed the possibility of immunising animals against diphtheria. In the same year, C. Fränkel (1890) and Behring (1890) began to work upon artificial immunisation against diphtheria in the Institute of Hygiene directed by Koch. While Fränkel directed his attention to immunisation by means of diphtheria cultures and endeavoured to obtain immunity by the injection of large quantities of broth cultures heated to 65° C., Behring, following the indications given by experiments on the treatment of animals by means of chemical agents, obtained another kind of immunity. Guinea-pigs which were inoculated subcutaneously with diphtheria cultures and injected immediately afterwards and on the three following days with certain quantities of 1—2% solutions of iodine trichloride, remained alive, whilst the control animals died within 24 hours. The survivors however suffered from limited local inflammation and necrosis of the skin. Animals, which had recovered their general health but still retained an open ulcerated surface, exhibited greater powers of resistance to infection than normal animals. After complete recovery two animals withstood injections of fully virulent diphtheria bacilli which killed control animals in 36 hours. In his first paper on diphtheria Behring (1890) made the following remarks on this subject:

> "A method of immunisation, which has not yet been used, consists in first infecting animals and then arresting the disease by therapeutic treatment. This method reminds one of the acquisition of immunity in man after convalescence from many infective diseases."

In regard to tetanus Behring and Kitasato (4. XII. 1890) made the following statement: "The immunity of rabbits and mice, immunised against tetanus, consists in the power of the cell-free blood fluid to render innocuous the toxic substance which the tetanus bacilli produce. This point of view had not been taken by any of the previous observers, some of whom had attempted to account for the phenomena by the theory of phagocytosis, others by the bactericidal action of the serum. The experiments on tetanus, which demonstrated the following facts explained, however, acquired immunity in diphtheria:

1. The blood of the rabbit immunised against tetanus possesses the property of destroying the tetanus poison.

2. This property can be demonstrated both in the blood and in the cell-free serum.

3. This property is of such a permanent nature that it is capable of acting when the blood is injected into other animals. The serum can, therefore, be used as a therapeutic agent.

4. Substances which destroy the tetanus poison are not found in the blood of susceptible animals, and when the tetanus poison is injected into susceptible animals it is possible after the death of the animal to recover the poison from the blood and other body-fluids."

Up to this time, owing to the fact that a sufficiently high degree of immunity had not been obtained, doses of the serum of immunised animals had been found to be incapable of protecting guinea-pigs against lethal doses of toxin. Further experiments carried out by Behring and Wernicke (1892) soon led, however, to the desired result. Behring (1891) communicated the results of these experiments to the 7th International Congress of Hygiene and Demography in London in the following words:

> "During the course of the last few months in conjunction with Dr Wernicke I have been able to demonstrate that the resistance of guinea-pigs against the specific diphtheria poison is increased with their resistance against the living bacilli, and that the blood of highly immunised guinea-pigs possesses outside the body the power of destroying the diphtheria poison, and further that guinea-pigs can be rendered immune by intraperitoneal injections of the blood of immunised animals, or, if infected, can be cured.
>
> To illustrate these points I will give the following examples:
>
> A guinea-pig immunised in October 1890 resisted in November and the beginning of December inoculations which killed control animals in 2—3 days. It suffered from a transient general illness, and infiltration at the seat of injection followed by necrosis.
>
> On the 11th December, 8 c.c. of blood were taken from the left carotid of this animal. The serum obtained from this blood was capable of partially neutralising lethal doses of poison. For example, whilst 1 c.c. of a solution of the poison when given alone killed a guinea-pig in two days, a guinea-pig injected intraperitoneally with 1 c.c. of the solution of the poison and 4 c.c. of serum died on the 8th day.
>
> The degree of immunity attained was not yet, however, sufficient to enable us to demonstrate with certainty the antitoxic action of the blood.
>
> This partially immunised animal received at the end of December another dose of a diphtheria poison which made untreated guinea-pigs ill but which was not fatal. No further injections were made for 3 months, but on the 1st April, 1891, the animal resisted a dose of diphtheria poison, which killed 11 other guinea-pigs within a few days.
>
> In May it was again, on two occasions, injected with a treble dose of a culture

of diphtheria, from which animals died in from 4—5 days. These injections produced no reaction and the animal had consequently an immunity of at least 6.

On May the 30th, 8 c.c. of blood were drawn from the right carotid. 4 c.c. of the serum, which was obtained, were mixed with twice the fatal dose of a toxin and the mixture injected into an untreated guinea-pig. This animal remained well and 5 days later it was found to be so far immune against an inoculation of diphtheria, from which the control animal died in 3 days, that it survived, though it exhibited a considerable local reaction (infiltration with subsequent necrosis). After the injection of a larger dose of the poison, given soon after, it died. A blood sample taken previously from the carotid did not exhibit any noteworthy antitoxic action.

The above highly immune animal received in June several doses of highly potent poison and gave no reaction.

Another guinea-pig, which was immunised in January of this year, had by May attained such a degree of immunity, that it exhibited no reaction when inoculated with twice the quantity of poison fatal for control animals in 4—5 days. From this animal 6 c.c. of blood were taken from the left carotid in July.

Two other guinea-pigs each received intraperitoneally 3 c.c. of this blood. One of them had been inoculated shortly before with a culture of diphtheria bacilli which killed two control animals in 3 days, and the other animal was inoculated 3 days after the injection of the blood. Both exhibited local infiltration and were ill for a short time, but did not die.

Having demonstrated in this fashion the possibility of obtaining in animals such an immunity against diphtheria that others could be immunised by injections of their blood, and even recover after infection had taken place, Wernicke and I immunised rabbits against diphtheria and used their blood with success in the immunisation and treatment of guinea-pigs."

The question of immunisation was thus substantially solved.

These fundamental observations were soon followed by the discovery of a large number of new methods of treatment, by means of which animals could be immunised against a fatal infection with bacilli or against fatal doses of poison. By the use of gradually increasing quantities of broth cultures or of diphtheria toxin active serums were always obtained from the animals.

Succeeding this, attempts were made to obtain sera containing antitoxin of the highest possible strength in such quantities that they could be employed in the treatment of human beings. Large quantities of blood serum could only be obtained from large animals, consequently sheep, goats, cattle, and eventually horses, were immunised on the same principles, and finally, by the use of very active toxins sera were obtained which contained large quantities of the active antitoxin. The preparation of the serum on a large scale was first undertaken by the aniline works of Meister, Lucius and Brüning at Höchst, and was introduced into practice in 1893.

Behring's fundamental discovery was the starting point for numberless investigations which have added greatly to our scientific knowledge. The production, actions, composition, alterations, and relations to antitoxin of the diphtheria toxin have been studied by many observers, especially Behring, Ehrlich, Roux and Yersin, Metchnikoff, Bordet, Wassermann, Arrhenius and Madsen, and the results have explained many of the complicated phenomena found in immunity against diphtheria.

Innumerable observations on the clinical value of the treatment by antitoxin, particularly the thorough investigations of Emil Roux, have expelled all doubts as to its efficacy. The mortality statistics, especially of the German Empire before and after the introduction of antitoxin, have demonstrated the enormous reduction in the death-rate in diphtheria brought about by the use of this remedy. In fact Behring's discovery conferred an incalculable benefit upon the human race.

The success of the therapeutic and prophylactic employment of diphtheria serum allows us to entertain the hope that we shall succeed, in conjunction with measures for isolation and disinfection, in again reducing, if not completely eradicating, the plague which has firmly established itself throughout the whole civilised world.

SECTION II.

CHAPTER II.

THE EPIDEMIOLOGY OF DIPHTHERIA.

By ARTHUR NEWSHOLME.

CONTENTS.

Introduction, p. 53.—Diphtheria in the prae-statistical period, p. 54.—Diphtheria in the statistical period, p. 55.—Death-rate in England and Wales, p. 56.—Statistical experience of other countries, p. 59.—Determining causes of epidemics and pandemics, p. 62.—Deficient rainfall and diphtheria, p. 64.—Brückner's weather cycles, p. 68.—Influence of soil, p. 69.—Damp houses, p. 70.—Analogy with scarlet fever, rheumatic fever, etc., p. 71.—Urban and rural diphtheria, p. 73.—Geographical distribution of diphtheria, p. 74.—Relation of the administration of antitoxic serum to epidemicity, p. 75.—Relation between prevalence and fatality, p. 76.—Natural variations in fatality, p. 80.—Seasonal incidence, p. 80.

WHEN a disease occurs like diphtheria in great epidemics with long inter-epidemic intervals, a study of its epidemiology must precede any accurate statement on the influence of season and still more of climate on its prevalence. To ascertain the geographical distribution of diphtheria we are still more dependent upon a knowledge of its epidemiology; for it would evidently be most fallacious to compare the amount of diphtheria in one community during an epidemic period with the amount in another community in an inter-epidemic period. Even when the average death-rates from diphtheria of two communities for a series of years are compared, error may arise; for it may happen, for instance, that the experience of one community during the period under examination comprises one and of the other community two epidemics, and that if a different series of years had been selected an opposite conclusion would have been drawn. For these reasons, when dealing with an epidemic disease like diphtheria, it is well to show in tabular form or plot out on a curve the annual death-rate for as long a series of years as can be secured. When different curves are then compared their relative heights can at once be judged by the eye, and the number of epidemic peaks embraced in each curve can at once be seen.

Our knowledge of diphtheria belongs to two periods, before and since statistical returns have been available. Our knowledge of the epidemiology of this disease would be defective, were our attention confined to the latter period; for, since the causes of death have been registered, only two great pandemics of diphtheria have occurred, and the basis of induction from the facts of these pandemics is correspondingly narrow. I propose, therefore, to consider briefly in the first instance the epidemiology of diphtheria prior to the pandemic of 1855–63, for which somewhat imperfect statistics exist in England. In most other countries statistics are of more recent standing.

Diphtheria in the Prae-statistical Period.—Diphtheria was known to the ancients, Aretaeus (*circa* A.D. 50) having described under the name of the "Syrian ulcer" the characteristic pharyngeal membrane and its extension to the larynx with consequent suffocation. The first great epidemic of diphtheria which can be definitely recognised was described by the Spanish doctors. It occurred between the years 1583 and 1618, under the significant name of "garotillo." Its ravages appear to have culminated in the year 1613, which was known as "anno de los garotillos." In 1618 it had spread to Naples, where in two years it claimed more than 5000 victims, chiefly children. Here it was known as "male in canna," or "affection strangulatoire." Between 1620 and 1642 it spread as far as Sicily and Sardinia, ending its cycle in 1642 with a second terrible recrudescence in Naples.

In the 18th century diphtheria spread beyond the two southern peninsulas to every part of Europe and to North America. Near the middle of the century France was invaded, first in detached places, but by 1750 nearly every part had become implicated. In 1749 Chomel described cases of diphtheritic paralysis in connection with the epidemic in Paris. Fagge states that in 1746 an epidemic occurred in Bromley, Kent. In 1749 an epidemic of "malignant ulcerous sore throat" appeared at Liskeard in Cornwall, of which Dr Starr contributed a description to the *Philosophical Transactions*. He fully described the false membrane and its extension to the larynx. About this time appeared Fothergill's *An Account of the Sore Throat* (London, 1748). Many of the old English descriptions leave it open to doubt whether scarlet fever with malignant sore throat was not being described. The confusion is not surprising, in view of the fact that diphtheria and scarlet fever are often epidemically prevalent together. In 1752 an epidemic of diphtheria was noted in New York. In 1775 diphtheria had become widely prevalent over the United States; and the death of Washington apparently was caused by it.

After 1770 epidemics of diphtheria which had been common in Central Europe gradually became rarer, and the disease was in partial abeyance through most of the first quarter of the 19th century. This long eclipse of the disease is especially interesting in view of the similar eclipse between 1855–63 and 1887. Between 1817 and 1826 diphtheria again became widely prevalent. In 1817 an epidemic occurred at Ashford in Kent, which was described by Burdon Sanderson as "an epidemic of malignant sore throat, which proved extensively fatal to children, was never accompanied with any eruption, and differed from scarlatina (Thorne, p. 15)." In Glasgow similar outbreaks occurred in 1819 and 1825. Between 1818 and 1821 occurred the well-known epidemic at Tours described by Bretonneau. A second epidemic occurred there in 1825–26; and in Bretonneau's treatise, which appeared in 1826, the term "diphthérite" was first used. This was changed by his pupil Trousseau to "diphtherie."

The first reference to climatic conditions affecting diphtheria which I have found was in the report of the Lancet Sanitary Commissioners in 1859. This report states that "while in the year 1825, a year remarkable for its extreme dryness, the communes north of Orleans were laid waste by diphtheria, it made as many victims in the damp and warm year, 1828, in the country south of Orleans."

Diphtheria in the Statistical Period.—Next came the great pandemic of 1855–63, which, so far as can be gathered from historical evidence, was greater than any outbreak since the 16th century, and differed from the latter in its wider distribution. As in all previous outbreaks in which the point is mentioned, the pandemic prevalence in 1855 onwards was preceded by numerous smaller outbreaks, like the sprinklings of rain before a downpour. Thus Netten Ratcliffe (1863) says that the first epidemic in England and Wales was in 1849–50 in Pembrokeshire. He mentions, however, that cases of diphtheria had been observed during 1848–49 in St Thomas's Hospital, London, in Herefordshire, Staffordshire, and near Yarmouth (Ratcliffe, 1863). In October, 1850, Dr Bennett read a paper before the Medical Society of London detailing cases of diphtheria in his practice. Netten Ratcliffe's remark as to "first epidemic" and the general impression that the

English cases of "Boulogne sore throat" in 1857 were a new disease, indicate that doctors were then unfamiliar with the disease, and throw much light on the invasive and intermittent character of its prevalence.

The various parts of Europe were invaded between 1855 and 1870. In 1859 it had become general throughout England. In 1856 a terrible epidemic occurred in San Francisco and in other towns of California, on which Dr J. V. Fourgeand wrote a monograph. Between 1855 and 1880 all parts of the United States had been invaded.

The preceding historical sketch is in rough outline, but this outline is needed if the characteristic epidemiology of diphtheria is to be understood. One further historical fact is needed in order to the correct interpretation of the statistics of diphtheria. In 1765 Dr Home of Edinburgh published a treatise on Croup, in which he accurately described the formation of a false membrane in the larynx and trachea, but unfortunately succeeded also in his attempt to convince the medical profession that angina maligna and croup were two distinct diseases, notwithstanding the prevalence for ages of the contrary view. In 1771, Dr Bard of New York published a treatise on Angina Suffocation, in which he supported the doctrine that the membranous process was the same whatever were the mucous membranes attacked. The treatise was republished in Paris in 1810 and was quoted by Bretonneau. It is unfortunate for the complete value of English statistics that Home's views were generally accepted, and that croup and diphtheria have continued to appear in the official reports as distinct diseases[1]. It is not suggested that every case of "croup" is—though doubtless the vast majority of such cases are—laryngeal diphtheria. That this is the present opinion of most medical practitioners is indicated by the diminishing proportion of croup to diphtheria in the national death-returns, as shown in the following table.

TABLE I. *Death-rate per million living from Diphtheria aud Croup in England and Wales.*

Periods:—	1858-60	1861-70	1871-80	1881-90	1891-1900	1901-03
Diphtheria	372	185	121	163	263	189
Croup	275	246	168	144	52	19
Croup per cent. of total Diphtheria *plus* Croup	43	57	58	47	17	12

---

[1] In the Nomenclature of Diseases drawn up by a Joint Committee appointed by the Royal College of Physicians and issued at the end of 1906, it is specially noted that "the use of the term 'membranous croup' as a synonym for 'laryngeal diphtheria' should be discontinued." In the Registrar-General's reports also, although the entry *Croup* remains, it is now defined as not including spasmodic or membranous croup. Membranous croup has been merged with diphtheria.

Between 1881 and 1890 a transference from croup to diphtheria began, which has gradually increased until in 1901–3 croup only formed 12 per cent. of the total as compared with 58 per cent. of the total aggregate deaths ascribed to diphtheria and croup in 1871–80. Thus at last we are approaching the view that croup and diphtheria are identical, which was taught by Bretonneau, was generally

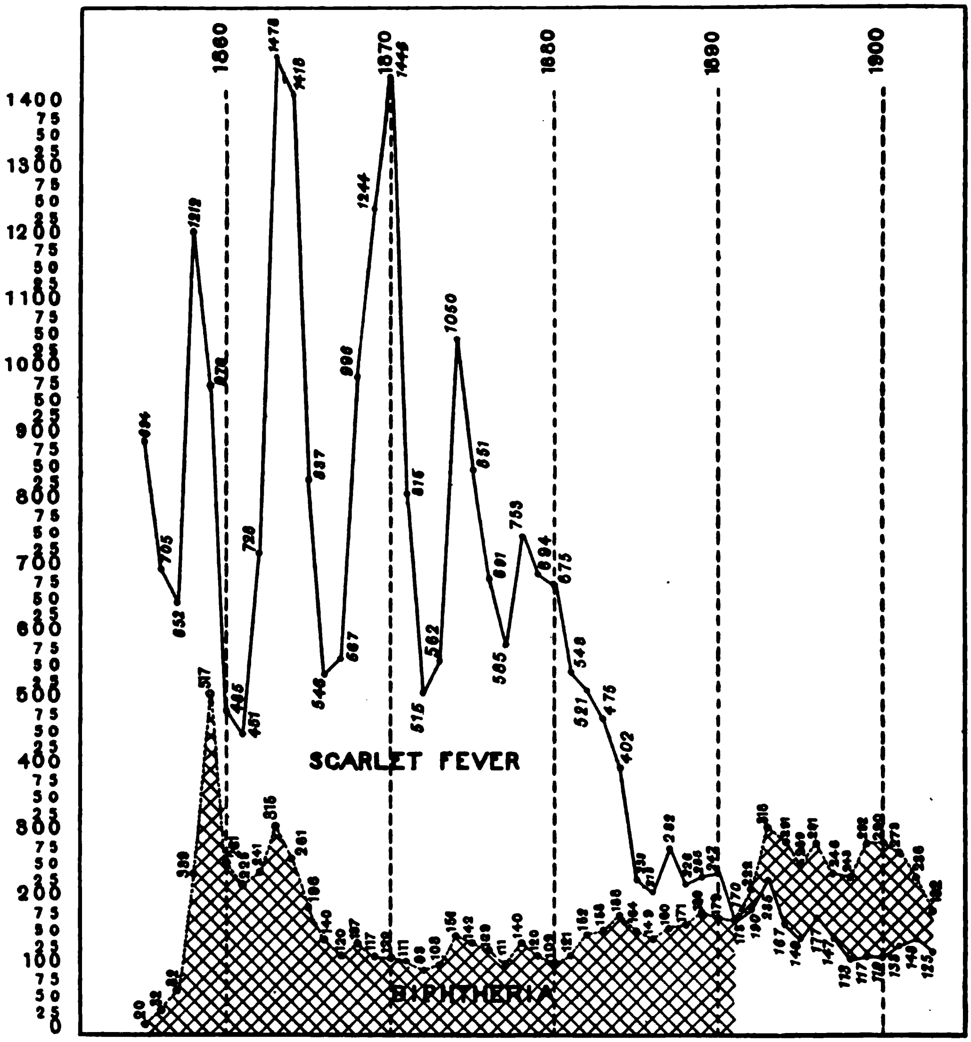


Fig. 1. Annual Death-rate per million persons living in England and Wales from Scarlet Fever and Diphtheria, 1855–1908.

accepted in the 16th and 17th centuries, and was known in the 1st century of the Christian era.

The recent epidemiology of diphtheria in England is set out in Fig. 1, which shows also the corresponding death-rates from scarlet fever. In view of the clinical affinities between the two diseases, and of the fact that they are frequently epidemic together, it is interesting to note how much more frequent as well as more fatal were the epidemics of scarlet fever than of diphtheria up to 1880, and how from 1889 onwards scarlet fever has become much less fatal than diphtheria. The knowledge derived from notification returns enables us to say that much of the diminished death-rate from scarlet fever is due to diminished fatality (case-mortality). There is no certain evidence of diminished fatality in diphtheria, apart from its treatment by antitoxic serum.

Fig. 1 represents the experience of England as a whole. Various parts of the country have suffered from epidemics of diphtheria in different years, and the preceding curve must therefore be regarded as a composite of these different experiences. The curve shows that during the period for which statistics are available two great epidemics have occurred, the first ranging from 1857–65 and the second culminating in 1893. Between these years diphtheria was endemically present to a less extent. To illustrate the gradual spread of diphtheria and the magnitude of epidemics in different towns the following examples are taken.

Table II. United Kingdom.

Death-rate per 100,000 *inhabitants from Diphtheria, in years of maximum and minimum Death-rate from this disease.*

	Maximum	Minimum	Years of observation
London	76 in 1893	8 in 1872	1871–1903
Croydon	95 in 1893	7 in 1897	1875–1905
Salford	106 in 1889	6 in 1873	1860–1905
Sheffield	133 in 1899	4 in 1884	1859–1904
Leicester	144 in 1900	2 in 1892	1857–1904
Bristol	54 in 1902	7 in 1890	1890–1904
Glasgow	60 in 1868	3 in 1860	1859–1904
Edinburgh	38 in 1889	9 in 1889	1880–1904
Dublin	26 in 1899	2 in 1889	1881–1903

The history of diphtheria in London in recent years also illustrates very well the rule that a large community is not entirely invaded in a single year, but suffers from repeated smaller outbreaks. The following figures in brackets give the maximum death-rate from diphtheria per 100,000 inhabitants in each metropolitan borough. Chelsea had a first maximum (58) in 1890, then an interval of much lower death-rate followed by a second maximum (117) in 1896. The maxima of Hammersmith were in 1891 and 1892 (75 and 74), of Fulham (99) in 1894, of many other metropolitan boroughs in 1893 ranging from 112 in Finsbury to 65 in Lambeth. Fulham had its maximum (99) in 1894; so had Paddington (76) and Camberwell (78) followed by a second higher maximum (101) in 1896. The detailed curves given elsewhere by the writer (Newsholme, 1898, p. 59) show that while one part of the metropolis was suffering severely from diphtheria, others were relatively exempt; these in their turn being invaded. These and similar facts to be culled from the experience of different parts of the country make it clear that diphtheria is a disease which creeps slowly from place to place, and that in each place months or even years may elapse before it takes firm root and begins actively to spread. Obviously climatic difference cannot explain these time-differences in the metropolitan boroughs, which must be in the main a question of opportunities for free personal inter-communication and infection. This of course does not exclude the possibility of auxiliary local factors special to some districts, nor of the operation of the wider causes determining an epidemic, to be subsequently discussed. The London experience is further interesting as showing that the first outbreak of the disease does not exhaust its local possibilities. A second outbreak or epidemic "relapse" occurred in recent years in at least fifteen out of the twenty-eight metropolitan districts examined by me. In the prae-statistical period similar experiences were observed at Naples and elsewhere.

Statistical Experience of Other Countries.—It is not proposed to pursue in detail the statistical study of diphtheria in other countries than England. The main facts can be set out in tabular form as follows, though curves showing the annual mass of the disease enable its incidence to be more easily followed.

TABLE III. FOREIGN CITIES.

Death-rate per 100,000 *inhabitants from Diphtheria, in years of maximum and minimum Death-rates from this disease.*

	Maximum	Minimum	Years of observation
London	76 in 1893	8 in 1872	1871–1903
Paris*	121 in 1877	10 in 1898	1865–1902
Rome*	122 in 1879	8 in 1895	1877–1895
Florence*	422 in 1871	23 in 1884	1866–1890
Antwerp*	217 in 1865	17 in 1896	1860–1896
Brussels*	118 in 1865	15 in 1876	1862–1896
Berlin	220 in 1883	31 in 1896	1869–1896
Buda Pesth*	194 in 1878	46 in 1895	1874–1896
Vienna	144 in 1878	19 in 1884	1865–1889
St Petersburg	154 in 1882	27 in 1893	1879–90 & 1892–95
Christiania	308 in 1887	1 in 1873	1860–1899
Stockholm	121 in 1893	6 in 1878	1861–1897
Copenhagen*	164 in 1891	15 in 1872	1844–1895
Boston	163 in 1881	51 in 1891	1861–1895
Providence	314 in 1877	8 in 1868	1868–1895
New York	208 in 1877	23 in 1873	1868–1893
St Louis	314 in 1877	8 in 1868	1868–1895

* Diphtheria *plus* Croup.

The facts in the above table when compared with those in Table II. and in Figs. 1 and 2 illustrate the general conclusion stated by me elsewhere (1898, p. 158), that *diphtheria is relatively a continental rather than an insular disease.* It may be mentioned that statistics of cities are given rather than of countries, partly because they are more easily obtained and as a rule more accurate, and partly because they are less composite, as regards epidemicity, than the statistics of countries.

It will be noted that the maximum death-rate from diphtheria has been without exception smaller in London than in any of the foreign cities compared with it. London is so great that its maximum death-rate from diphtheria may also be regarded as a "composite"; it may be added, therefore, that the maximum death-rates of the majority of English towns is much lower than that of the European and American cities enumerated in Table III., the experience of Sheffield and Leicester being exceptional. It should also be noted that these foreign cities, in proportion to their magnitude, probably share with London the "composite" character of their maxima. Diphtheria caused much smaller mortality in Ireland than in England, and in New Zealand than in Australia. These facts justify the conclusion that so far as can be judged from the past history of diphtheria and from the more recent statistical experience concerning it, diphtheria has always committed more ravages among communities living in continents than among insular communities. This might be explained on the score of freer personal communication, but the relative experience of Australia and New Zealand scarcely supports this interpretation; and the fact that the distinction between continental and insular capitals, e.g. between New York, Berlin, and Paris on the one hand, and London on the other, continues to hold good in these days of rapid inter-communication is against any explanation based on relative ease of introduction of infection. We know that its insular position has not prevented London from suffering very severely from influenza.

Further conclusions as to the international prevalence of diphtheria can be drawn from Tables II. and III. Not only is it more a continental than an insular disease, but a study of the annual death-rates for a series of years (of which Fig. 3 on page 76 may be taken as an example) shows furthermore that much more than in English towns there has been, both in American and European cities, *a large mass of diphtheria constantly endemic*. Diphtheria in these latter cities,—and so far as available evidence extends in the countries of which they form a part,—partakes much more of the nature of an endemic disease than in England. The country having in the past the smallest amount of diphtheria endemically present has been Ireland, the next smallest being England and New Zealand, Scotland coming after these. Allowing for the fact that the Paris statistics do not separately state diphtheria and croup, it would appear that Paris and Berlin occupy a supreme position in Europe as regards the amount of endemic diphtheria. Munich and Hamburg are not far behind, while in Brussels it is a less heavy cause of death. Italian cities also suffer severely. In Scandinavian cities there has been a very heavy death-rate from diphtheria; but the disease has prevailed chiefly in an epidemic form. Returns from Boston, New York, Chicago, and Montreal render it probable that there has been even more endemic diphtheria in these cities than in Berlin or Paris.

The history of the disease and the statistical evidence appear to indicate that *diphtheria spreads slowly* from community to community. Whether there are special indigenous foci of the disease is open to doubt. The hypothesis that such foci exist appears superfluous, as it is seldom completely absent from large communities in which the point can be tested.

Superadded to endemic diphtheria ***epidemics and pandemics of the disease occur cyclically.*** The following list gives some of the maximum epidemic years in different countries during the period for which statistics can be obtained. The list is merely illustrative and cannot fully display the facts of prevalence, for which curves of annual death-rate would be required.

TABLE IV. *Maximum Epidemic Years in*

		1860	1870	1880	1890
A. AMERICA.					
Boston		1863–64	1875–76	1880–81 1889–90	1894
Providence			1877–78	1886———90	
New York			1876–78	1880–82 1886—88	1893–4
Chicago		1860–65 1869–70	1876 1879–80–81	1886–87 1890	
B. EUROPE.					
London	1858	1861–63	1875–76		1892–96
Paris		1865–66	1872–73–75–77 1879–80–81–82		
Berlin		1869–70	1875–76	1882–84	1893
Christiania		1861–62		1885–90	
C. AUSTRALASIA					
Melbourne	1858–61	1867–68	1873–74	1889–90	
Sydney		1863–64	1875	1889	

The intervals between the cycles of epidemic prevalence vary greatly. A study of the continental figures shows shorter intervals than those for islands, and as already explained the difference between epidemic and inter-epidemic death-rates is less marked in continental than in insular climates. In London when diphtheria became epidemic in 1857, it was thought to be a new disease. It did not again become epidemic until 1875 and then feebly; and it was not until 1893 that the third (the second great) epidemic of which statistical records are extant reached its maximum. The possible causes of these longer cycles will now be discussed.

Determining Causes of Epidemics and Pandemics.—The presence or absence of the Klebs-Loeffler bacillus cannot be regarded as exhausting the explanation of the occurrence or non-occurrence of an epidemic of diphtheria. We have seen that in most if not in all large communities a certain amount of diphtheria is permanently endemic. In smaller communities most physicians must have had experience of the introduction of diphtheria without its spread, although opportunities for such spread existed which, so far as could be judged, were as great as those which in other instances were followed by the spread of infection. It seems clear that under certain circumstances influences inhibiting infection or possibly diminishing the "carrying power" of the contagium itself are operative. It is conceivable that at the end of an ill-controlled epidemic the failure of infection to continue to spread is associated with the intra-corporeal development of antibodies caused by a slight unrecognised attack ("missed cases"), or derived from Klebs-Loeffler bacilli which have invaded the mucous membrane without causing any illness ("carrier cases"). This may explain the dying out of an epidemic before all the children not naturally insusceptible and not temporarily protected by a prior attack have had diphtheria. Are we to suppose that this immunity endures for a number of years, and that when it is exhausted in a population the next epidemic cycle occurs? Or does the next cycle occur when a sufficient number of younger children not born at the time of the previous cycle have accumulated to form the necessary links in the chain of infection? The first supposition is negatived by the imperfect and evanescent protection given by one attack of diphtheria, as evidenced by the occurrence of relapses and second attacks. Nor does the second supposition appear very probable, except in those communities in which an epidemic has been allowed to run rampant, even were it assumed that the protection by one attack were more lasting than experience indicates.

In view of the preceding considerations it is improbable that the relative freedom of a population from diphtheria for a long period can be ascribed to a general insusceptibility of that population. And yet in the majority of large communities a certain amount of diphtheria is always present, or is frequently introduced without active spread. It appears to follow that something more than importation of infection, and something beyond the dying out of general insusceptibility, are required to explain why in certain years diphtheria becomes epidemic, and still more to explain why in certain years it becomes pandemic. It is highly improbable that in pandemic periods a widespread alteration in the personal susceptibility of the populations of continents will almost simultaneously have occurred. A more probable explanation of epidemicity lies in some change or changes in the external conditions of life. If such external changes are found always to coincide with great epidemics of diphtheria, the means by which they produce such epidemics will require discussion at a later point.

Deficient Rainfall and Diphtheria.—One such external condition has been shown by me to be uniformly present,—in all instances in which the point can be statistically tested,—whenever great epidemics of diphtheria occur. The relationship is the more striking, because it was unsuspected; the general teaching. having been that diphtheria is most prevalent in damp houses and on damp and low-lying soils, the condition of which would obviously be bettered during a succession of dry years. This external condition is summed up in the general conclusion that "diphtheria only becomes epidemic in years in which the rainfall is deficient, though in some years the excess of diphtheria in dry years is but small. There are no instances of a succession of wet years in which diphtheria was epidemic." Only two great epidemics have occurred during the period for which corresponding statistics of rainfall and diphtheria exist. The induction therefore has a very limited basis so far as any one locality is concerned. As, however, it is found that during the same period the coincidence between a succession of dry years and epidemic diphtheria occurs in every civilised part of the world for which statistics exist, and that in these different parts epidemics of diphtheria never begin in wet periods, the induction is one to which a much higher degree of probability attaches.

The instance of London may be taken. Its experience is set forth year by year in Fig. 2, and in Table V. this experience is summarised and compared with the annual rainfall for the corresponding years or groups of years. If the table and the figure are studied together no difficulty of interpretation will arise. It will be noticed that in the figure each year's relative death-rates are given separately, and that in the table years of maximum death-rate are also given separately. It must also be remembered that the earlier London epidemic of 1859 onwards is almost certainly understated, owing to confusion with scarlet fever, which had up to that year been entered in the tables along with diphtheria.

TABLE V. *Rainfall and Diphtheria in London.*

(Mean Annual Rainfall and mean Death-rate from Diphtheria for the whole period = 100.)

	1854-8	1859	1860	1861-64	1865-70	1871-80	1881-88	1889	1890-92	1893	1894-1900	1901-0
Annual Rainfall	85	108	134	88	109	111	98	98	97	84	93	101
Annual Diphtheria	(no record)	**120**	74	**104**	57	52	100	**166**	**161**	**323**	**195**	98

No diphtheria returns for London are available prior to 1859, but we know that for several years before 1859 "croup" was excessively fatal, and so probably was diphtheria. The drought in 1858 and 1859 was so great, and the river Thames ran so low, that the stench from it was regarded as foreboding much fever. It will be noted that in the wet year 1860 a lull in the epidemic occurred. The great epidemic of recent

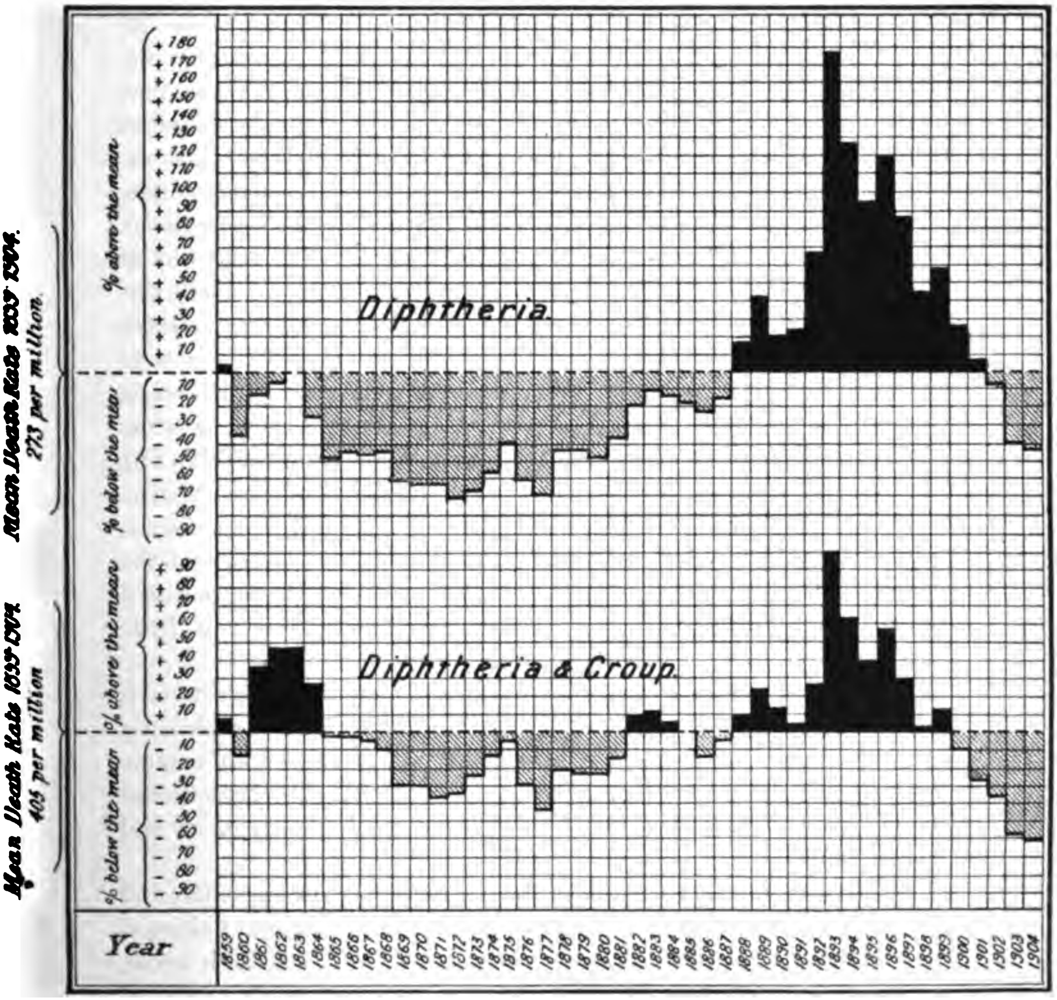


Fig. 2. Annual Death-rate per million persons living in London from Diphtheria and from Diphtheria and Croup, stated in proportion to the mean Death-rate for the whole period[1].

---

[1] From Sir Shirley Murphy's *Annual Report on Public Health, County of London*, for 1904.

years was associated throughout with deficient rainfall. Other instances of the same relationship are given by me elsewhere (1898, pp. 142—161).

If the association between deficient rainfall and epidemic diphtheria involves some element of causation, it ought to be visible not only when the rainfall and diphtheria of a given community are compared in time; but also when place is compared with place. When the comparison is practicable, it is found that in a given country the amount of diphtheria is greatest in the parts having the smallest rainfall, and the amount of diphtheria in different countries bears an inverse relationship to their rainfall, though the relationship is not one of simple proportion. The first point is well seen in Australia. The total mass of and the epidemic death-rate from diphtheria are much less in Sydney with an average annual rainfall (for 1840–95) of 49·7 inches, than in Melbourne with an average annual rainfall (for 1857–95) of 26·5 inches; while Adelaide with an average annual rainfall (for 1839–94) of 21·4 inches has a larger amount of diphtheria than either Melbourne or Sydney. The same phenomenon is seen when Bristol with a heavy rainfall is compared with Croydon, or when Dublin or Belfast is compared with any large English town (Table II.). New Zealand gives an interesting example of the same phenomenon. Canterbury, N.Z., has much more diphtheria than any other district of New Zealand. It is less hilly and broken up than the rest of the country, and it has an average annual rainfall of 25 inches as compared with 37 to 41 inches in the districts in which diphtheria is little prevalent.

Two other considerations support the induction that diphtheria is a disease which prevails inversely with the rainfall. First, diphtheria is a rare disease in tropical climates and is not known to assume epidemic proportions in them. Tropical countries are characterised not only by excessive heat, but also by excessive humidity of air and copious rainfall.

Secondly, as already shown, diphtheria is relatively more a continental than an insular disease. It is immensely more rife in the great cities of the European and American continents than in British and Irish towns, and in Australia than in New Zealand. A continental differs from an insular climate in its greater variations in temperature and in rainfall; and the rainfall may be taken as an index of these variations. The general law is that "the intensity of the oscillations of rainfall increases with the continentality of the region" (Waldo, 1893, p. 409). Not only is there greater oscillation but a greater difference in the actual amount of rainfall, that of Berlin and Paris for instance being much smaller than that of London.

The relationship between deficient rainfall and excessive diphtheria needs to be further tested by a search for (1) periods of exceptional deficiency of rainfall without the occurrence of epidemics of diphtheria; and of (2) wet periods in which such epidemics began. Neither of these points can well be tested in continental cities because the amount of endemic diphtheria is so great as to obscure the magnitude of epidemics. In England in 1873–74 there was deficient rainfall in London with only slight excess of diphtheria; as pointed out by me elsewhere (1898, p. 152): "diphtheria only becomes epidemic in years in which the rainfall is deficient, and the epidemics are on the largest scale when three or more years of deficient rainfall immediately follow each other." In 1873–74 the deficiency of rain was much less protracted than that which characterised the two great epidemics in the sixth and ninth and tenth decades of the 19th century. When I previously wrote on the relationship between deficient rainfall and diphtheria, Sheffield and Leicester appeared to be examples of towns in which the relationship did not appear. The details given below show, however, that they are examples in which what may be described as the local "reaction" was merely somewhat slow in appearing. In this respect they closely resemble Christiania, the details of which are given elsewhere (1898, pp. 123 and 148). The extent of the comparison is limited in the instances given below by the duration of the record of rainfall.

TABLE VI.

SHEFFIELD.

	1880-86	1887-93	1894-98	1899	1900	1901-04
Annual Death-rate from Diphtheria per 100,000	20	30	23	**188**	**182**	28
Deviation of the Rainfall of each year from mean Annual Rainfall for 1880–1904 stated as 100 (=30·1 inches)	112	87	98	98	109	102

LEICESTER.

	1885-86	1887-93	1894-98	1899	1900	1901-03
Annual Death-rate from Diphtheria per 100,000	6	8	24	**108**	**144**	34
Deviation of the Rainfall of each year from mean Annual Rainfall for 1885–1903 stated as 100 (=22·9 inches)	135	92	90	97	118	92

In both of these towns as in Christiania the epidemic began near the end of a series of dry years and persisted into the next wet year.

The instances above quoted indicate that the local incidence of diphtheria in relation to rainfall is not a simple one, the local reaction to the influence of protracted years of deficient rainfall varying both in rapidity and in amount. I have been unable to discover any example of a widespread epidemic of diphtheria affecting a number of communities in a wet year or during a period of wet years; and the experience of a large number of communities justifies the general conclusion that "diphtheria is nearly always at a very low ebb during years of excessive rainfall, and is only epidemic during such years when the disease in the immediately preceding dry years has obtained a firm hold of the community, and continues to spread presumably by personal infection."

It is clear that in stating that great epidemics of diphtheria are associated with dry periods of years we do not exhaust the causation of this disease. We only state one of the conditions,—probably a chief one,—which is necessary to secure the successful invasion of large populations by the specific infection of diphtheria. The use of the word "necessary" in the last sentence is limited by the extent of the experience, hitherto without exceptions, on which the conclusion is based. It may be that when the subject is further investigated in the light of more extended statistics, some index other than annual rainfall will more accurately represent the undoubted relationship between dry periods and epidemic diphtheria. For the present the use of this ratio appears to bring us a step nearer to a rational explanation of *le milieu épidémique* or, as the old physicians described it, the "epidemic constitution" which appears to be a necessary antecedent of a great epidemic.

Brückner's Weather Cycles.—An interesting light is thrown on the preceding discussion by the elaborate observations made by Professor Brückner of Berne on the world's rainfall. His data deal chiefly with the years 1830–85 and are summarised in the following table:

TABLE VII.

	Periods of Rainfall				
	Deficiency	Excess	Deficiency	Excess	Deficiency
Europe	1831–40	1841–55	1856–70	1871–85	—
Asia	1831–40	1841–55	1856–70	1871–85	—
Australia	–45	1846–55	1856–65	1866–75	—
North America	1831–40	1841–55	1856–65 (71–75)	1866–70 (76–85)	1876–85
Central and South America	1831–45	1846–60	1861–75	1876–85	—

Dr Waldo (1893, p. 411) after giving the above table adds a forecast which has been fulfilled: "it would appear probable that we are now entering upon another period of very low rainfall during 1890–95." It is impossible exactly to compare such average results with the diphtheria death-rates for particular towns in different countries, which have not been constructed by a similar system of averaging. But with this proviso the above table when compared with the years of maximum and minimum death-rates from diphtheria in tables II., III., and IV. is most suggestive. The exceptional period of deficiency of rainfall in North America in 1876–85 fits in with the exceptional American maxima of diphtheria during the same period.

The Influence of Soil on Diphtheria.—There has been fairly dogmatic teaching on this subject. Thus Dr Copeman (1892) says: "Many districts which, although usually dry, are liable to occasional floods, are remarkably free from the disease, so that it appears that a persistent impregnation of the soil with moisture is of more importance than fluctuations in the height of the ground water, particularly if these have any considerable range." These remarks appear to imply an antithesis between dry soils and diphtheria, as well as a causative relationship between persistently moist soils and diphtheria, on both of which issues I must give an exactly opposite opinion.

The late Sir Richard Thorne Thorne (1891, p. 17) while agreeing that "the broad geological features of a district have no known influence on the development or the diffusion of diphtheria," states that he can hardly agree with Hirsch that "the assumption that *conditions of soil* have some influence in the development of diphtheria, or on its epidemic diffusion, is one that has no warrant." The essence of Dr Thorne Thorne's teaching is contained in the following paragraph: "Soil, and especially *surface soil,* when considered in connection with relative altitude, slope, aspect, and prevailing rainfall, has, I believe, concern in the maintenance and diffusion of diphtheria, and has very possibly some relation with its beginnings. Speaking generally, I think that the experience of careful investigations extending over a number of years is to the effect that where a surface soil is, by reason of its physical constitution and topographical relations, such as to facilitate the retention of moisture and of organic refuse; and where a site of this character is, in addition, exposed to the influence of cold wet winds, there you have the conditions which do tend to the fostering and fatality of diphtheria, and which also go to determine the specific quality of local sore throat." And yet we have seen that so far as the two great English epidemics of diphtheria are concerned,—and the evidence from them is confirmed by international experience,—diphtheria prevails chiefly when a series of dry years are concerned. In such dry periods the influence of damp soil as described in the preceding quotations would be at a minimum. The conclusion that damp soils favour diphtheria may be based on the impression derived from "experience." It is possible that an accurate investigation would show a greater prevalence of diphtheria on low-lying and damp than on high and relatively dry soils. But it would not follow that any causative relationship between the two existed unless either the observation were confirmed on a very large scale, or failing this, correction were made for differences of age distribution of populations, and allowance were made for the fact that people living on low-lying soils are usually poorer and therefore more exposed to infection than those who are able to live in more favoured localities.

Before stating the only conclusion which can be formulated as to the relation between soil and diphtheria, it is convenient to mention here the supposed relationship of damp houses to diphtheria.

Damp Houses and Diphtheria.—In 1878 Dr Thursfield published papers illustrating the way in which diphtheria hung about certain damp houses, and this observation has been confirmed by others. Before, however, any causative relationship could be based on such localised experience it would be necessary to know the proportion of such damp houses, both among those invaded and among those not invaded by diphtheria. If the majority of houses in a given district are damp, the association of dampness of house with recurrent diphtheria can have little significance. Since the above observations were published, our knowledge of protracted diphtheria has become more accurately determined. I have, for instance, had cases of diphtheria in which Klebs-Loeffler bacilli, proved by inoculation to be virulent, have persisted in the mucous secretions of patients otherwise apparently well for 102, 110, and 170 days respectively, and more protracted cases have been described. It seems more likely that in the cases described by Dr Thursfield and others we have to deal with human carriers of infection, in whom a slight catarrh causes a recrudescence of dormant infection, rather than with the saprophytic persistence of Klebs-Loeffler bacilli in the house. Even so the house may have an influence in securing recrudescence. Dampness favours sore throat and catarrh and may thus open the way for re-invasion by personally stored diphtheria bacilli. A careful distinction must be drawn between the influence of a protracted dry period and that of damp houses or of the rainfall say of a single week. The latter conceivably may favour diphtheria as well as the former, the one acting on the individual, the other in some unknown way. At present we are only in a position to say that in view of the inverse relationship between rainfall and diphtheria the only condition of soil that can favour diphtheria is one of exceptional dryness and warmth. The analogy of other diseases may help in arriving at a conclusion as to such a connection.

Analogy with Scarlet Fever, Rheumatic Fever, etc.—It has been shown independently by Drs Gresswell and Longstaff that the yearly mortality from scarlet fever is inversely to the amount of rainfall. Dr Gresswell (1890, p. 192) has further suggested that "not only the rainfall of the year, but also that for prior years, has influence on scarlatina." Dr Longstaff (1891) showed that the chief increases in the death-rates from scarlet fever, erysipelas, puerperal fever, and rheumatism in England and Wales occurred in years of deficient rainfall. In the Milroy Lectures for 1895, I showed by elaborate mortality and sickness statistics derived from the general mortality experience of different European countries, from the general notification experience of Scandinavian countries, and from the experience of large general hospitals in England and other countries, that rheumatic fever is an epidemic disease, of which widespread epidemics occurred in years in which there was always an exceptional scarcity of rainfall. It was also noted that "the effect of rainfall is not produced immediately. It takes time to develope: and it is warrantable to assume that the influence of deficient rainfall is exerted as the result of its effect on the subsoil, this effect usually showing itself by a marked lowering of the ground-water." The lowness of the ground-water, of which examples were given, was regarded merely as an index of certain conditions of dryness and temperature of the subsoil which greatly favour the growth of the contagium of rheumatic fever.

Arguing from the analogy of rheumatic fever,—while bearing in mind that in rheumatic fever and scarlet fever the response between dry years and epidemics was more prompt than in diphtheria,—I formulated the following working hypothesis of the causation of diphtheria. The specific micro-organism of this disease has a double cycle of existence, as have apparently the specific micro-organisms of enteric fever, erysipelas, scarlet fever, rheumatic fever, etc. One phase is passed in the soil, another in the human organism. One is saprophytic, the other parasitic, though it is not contended that there is any regular alternation between these phases. It is not strange, therefore, that the epidemic prevalence of all the above diseases is favoured by deficient rainfall, if this is sufficiently long continued. This deficient rainfall implies a low subsoil water, and a subsoil above the level of this water, which is relatively dry and warm, probably the *optimum* conditions for the saprophytic life of the above pathogenic micro-organisms. The causes of the transition of the diphtheria bacillus from the saprophytic to the parasitic phase of life may be surmised both as regards (*a*) season and (*b*) years of special epidemic prevalence. Diphtheria is most prevalent in autumn and in the early winter months, when the optimum temperature and the optimum degree of humidity of the soil are rapidly disappearing or have departed. It is also most prevalent after the wet weather occurring in or immediately following exceptionally dry years. Both these conditions tend to raise the ground-water and to drive out any pathogenic micro-organisms from the soil.

The preceding working hypothesis may appear to give undue importance to climatic conditions as contrasted with personal infection. There is no reasonable doubt that personal infection is the chief means by which diphtheria is spread. Personal infection does not, however, explain why in some years diphtheria, although present in a district in an endemic form, does not spread; while in another year, in which only the same opportunities of personal infection occur, it becomes extensively epidemic. Still less does it explain the occurrence of widely scattered epidemics and even pandemics in certain years. To explain these the operation of wider general causes must be presupposed. It might be that the susceptibility of entire populations to the infection of diphtheria increases at times, though this is improbable; or it might be that the diphtheria bacillus under certain conditions becomes more actively virulent and infective—more remote from its saprophytic phase of life; and thus, persons who can resist the ingress of the feebler, fall victims to the more powerful micro-organism. The latter is probably the correct hypothesis; and the evidence already given clearly points to the conclusion that of the external cultural conditions leading to increased virulence of the diphtheria bacillus and greater readiness for assuming a parasitic life, exceptional deficiency of rainfall and consequent exceptional deficiency of moisture in and exceptional warmth of the subsoil form an essential part.

The working hypothesis given above is unproved. It may not be supported by further investigation. It is possible that dry periods favour epidemic diphtheria otherwise than I suggested in the preceding remarks written in 1897. They may act by increasing the invasion of the mucous membranes of a large portion of the population by auxiliary organisms which favour the pathogenic action of the Klebs-Loeffler bacillus; or some other explanation may be forthcoming. But the fact out of which these theories grow remains. Diphtheria prevails epidemically on a large scale only during dry periods of years; and the same relationship between these two phenomena exists as was previously shown by Dr Longstaff to hold good for erysipelas, scarlet fever, etc. Minor differences however are shown, the occurrence of a pandemic of diphtheria appearing to necessitate a more protracted continuance of deficient rainfall than is required to favour the epidemic prevalence of the other diseases named above.

Urban and Rural Diphtheria.—Longstaff (1889) in an important paper examined the diphtheria statistics of England and Wales in the 26 years from 1855 to the end of 1880, classifying populations, according to their density of population, into Sparse, Medium, and Dense. The death-rates from diphtheria in each of these three groups of population are shown in the following table:

TABLE VIII. *Death-rates from Diphtheria per million living in different parts of England and Wales.*

	1855-60	1861-70	1871-80
Dense Districts	123	163	114
Medium ,,	182	164	125
Sparse ,,	248	223	132

In the first period there was most diphtheria in the sparse districts; in the second period the same result is seen but less markedly; and in the third period, which was almost entirely a non-epidemic period, there was relatively small difference between the three groups. Since 1880 the incidence of diphtheria on towns has become more marked, and diphtheria which, as the late Sir Geo. Buchanan said, "has always displayed a marked tendency to prevail in sparsely-populated districts rather than in centres of population" is undergoing a change of distribution by which "the chief urban districts seem to be approaching nearer than before to rural districts in their rate of suffering from this disease."

The difficulty as already pointed out in all such local comparisons consists in the unequal distribution of epidemics. The same difficulty arises if counties of England are compared. Thus taking the death-rates for 1898–1902 published in the Registrar-General's Annual Report for 1903, the death-rate per million population from diphtheria in 1898–1902 includes the following instances of high death-rates in "dense" counties, viz. 353 in the West Riding, 524 in Leicestershire, 390 in Staffordshire and 290 in Lancashire, and the following instances of low death-rates in sparse counties, 116 in Oxfordshire, 129 in Cornwall, 150 in Dorset. The highest death-rate in a sparse county was 298 in Buckinghamshire, the lowest in a dense county 149 in Nottinghamshire. In Norway the inverse relationship between density of population and death-rate from diphtheria is not shown when a long period is taken. In Christiania in the period 1860–93 the average death-rate from diphtheria was 800, in Norway as a whole in 1860–91 it was 400 per million living. The subject must be left thus undecided. At the present time diphtheria is undoubtedly a heavier cause of mortality in urban than in rural districts. If the position was the reverse in 1855–60 some light might thus be thrown on the possible source of diphtheria. In forming an opinion we have to remember that between 1855 and 1860 there was much confusion between croup and diphtheria. This is clearly shown in Shirley Murphy's diagram of the experience of London (Fig. 2). Longstaff's figures only deal with diphtheria. The statistics for London in 1855–60 would be a heavy factor in determining the low relative position of the dense districts as regards diphtheria mortality; but there is no evidence that in other districts there was less confusion of diphtheria with croup than in London.

Geographical Distribution of Diphtheria.—The preceding account of the epidemiology of diphtheria has been concerned throughout, though indirectly, with its geographical distribution, and we have seen how difficult it is, when concerned with a disease of intermittent prevalence, to give a quantitative statement of its prevalence. Statistics of localised districts would be apt to mislead, and any conclusions based on them as to the influence of soil or other local conditions would need to be carefully checked as to the relative epidemic experience of the districts under comparison, and carefully corrected for age-distribution of population. Certain broad results as to geographical distribution seem, however, to be well established by the preceding data and may be here summarised. Diphtheria is almost absent from tropical regions.

It is much more largely a continental than an insular disease. In single countries it is most prevalent, and the death-rate caused by it is greatest in those parts of these countries in which there is least rainfall; and in all parts it is most prevalent in dry periods. It is least prevalent in Ireland, and less in the West of England and in Wales, with the possible exception of some parts of Wales, than in the rest of England. It is similarly less prevalent in New Zealand than in Australia, and less prevalent in New South Wales than in Victoria.

Relation of the Administration of Antitoxic Serum to Epidemicity.—As antitoxin is given with varying promptitude and to a varying extent in different communities, it is obvious that an additional element of confusion has arisen in comparing statistics of deaths from epidemic diphtheria, in relation to all the problems hitherto considered and in relation to the subject of the next paragraph. The adoption of the antitoxin treatment of diphtheria may be said to date from Roux's address to the International Congress of Hygiene and Demography, Budapest, 1894, the rapidity and extent of its adoption since that date having varied greatly in different communities. The curves of death-rate from diphtheria in Paris (Fig. 3) and in London (Fig. 2) may be considered in relation to the treatment by antitoxin. Doubtless very many lives have been saved by its means. This is clearly shown by the statistics of cases treated in hospitals and particularly of cases of laryngeal diphtheria requiring operation. The entire clinical view of the disease has been altered by the happy results obtained by means of the early administration of antitoxin. These clinical facts have a bearing on the epidemicity of diphtheria in so far as the death-rate from it has been lowered by the therapeutic use of antitoxin. A marked influence in lowering the death-rate from diphtheria must be credited to antitoxin both in Paris and London, probably more in the former. That the decline in the death-rate from diphtheria since 1894 is not solely and possibly not chiefly due to this cause is evident when the course of the curves in Figs. 2 and 3 is studied. Thus in London a marked decline was already visible in 1894, which has since continued at an increasing ratio after 1897. In Paris a considerable decline began in 1891, and the general slope from 1880 onwards leaves no doubt that before antitoxin was introduced a decline of the death-rate from diphtheria was already in progress. On the other hand the amount and rapidity of the decline, reaching a minimum in the years 1895–1900 which was much lower than the minimum in 1870, are probably due in large measure to the popularity in Paris of treatment of diphtheria by antitoxin. Mixed causes have evidently been in operation. Antitoxin decreases the severity of diphtheria and lowers its fatality; but beyond this cyclical causes have been operating to produce a great reduction of the death-rate from diphtheria both in London and in Paris.

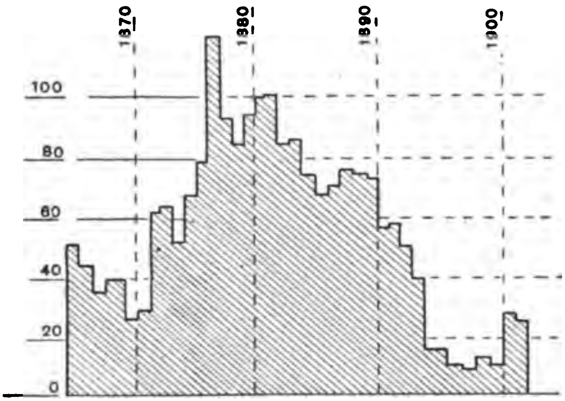


Fig. 3. Annual Death-rate per 100,000 persons living in Paris from Diphtheria *plus* Croup, 1865-1902.

Relation between Prevalence and Fatality of Diphtheria.—It cannot be said that exact data for the study of this problem exist. The difficult questions as to whether the age-distribution of attacks is the same during and between epidemics, and as to whether the notification of cases is equally complete throughout need prior determination. Bacteriological diagnosis has doubtless led in recent years to the notification of many cases formerly overlooked. Subject to these sources of possible error, Figs. 4 and 5 are interesting illustrations of this problem. In Norway a national system of registration of sickness has existed for many years. During 1860–91 in that country the mean annual sickness-rate from diphtheria was 2·15 per 1000 of the population, and the mean annual death-rate ·40 per 1000. In Copenhagen a similar system is in force, and during 1855–95 the mean annual sickness-rate from diphtheria was 5·88 per 1000, the death-rate ·78 per 1000.

The curves in both figures 4 and 5 show a remarkably close relationship between prevalence and mortality, and therefore a fairly uniform fatality (which is the proportion between prevalence and mortality). Certain minor changes are visible. In Norway during the greater part of the inter-epidemic period there is some evidence of greater fatality.

But this may have been owing to the fact that at such times the disease was less generally recognised and notified than when its epidemic prevalence would constantly obtrude it on the doctor's attention. In Copenhagen the records, if trustworthy, show an increased fatality in the epidemics culminating in 1865 and in 1879; but during the epidemic culminating in 1890 the cases increased in a higher proportion than the deaths.

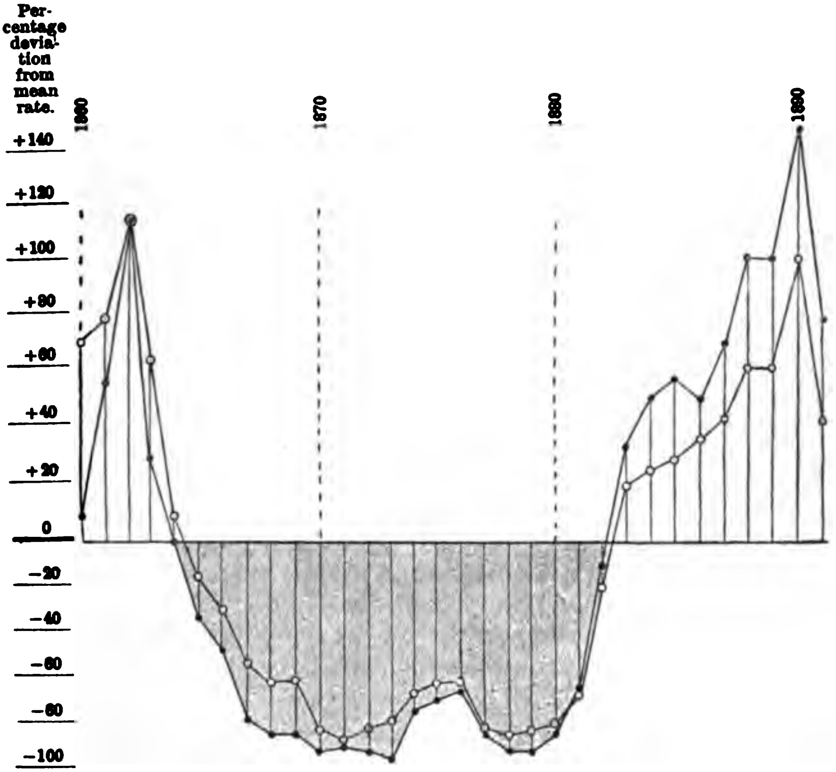


Fig. 4. Norway.—Annual deviation of the sickness-rate (o–o–o–o) and death-rate (●–●–●–●) caused by Diphtheria from the mean rate for the entire period 1860–91.

A careful study of Fig. 6, copied by permission from Sir Shirley Murphy's Annual Report to the London County Council for 1904, enables the same problem to be studied with the addition to the problem of variations in fatality associated with the different months of the year. The seasonal variation of fatality of scarlet fever and diphtheria in London during the eight years 1891–98 is further set out

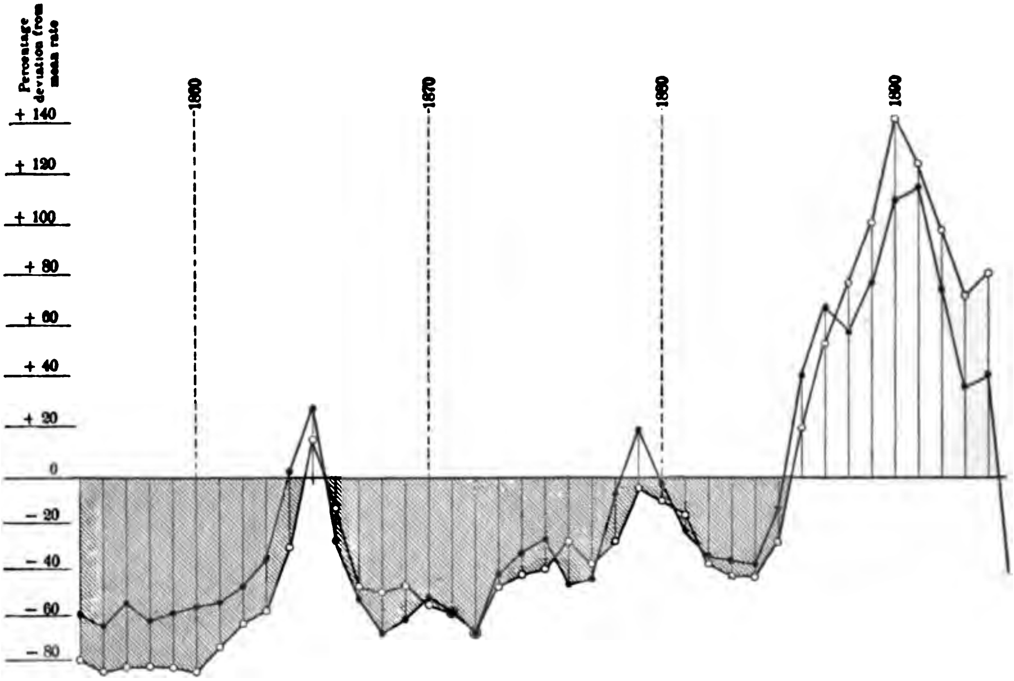


Fig. 5. Copenhagen.—Annual deviation of the sickness-rate (o–o–o–o) and death-rate (•–•–•–•) caused by Diphtheria from the mean rate for the entire period 1855–94.

in the following table and remarks derived from Sir S. Murphy's report for 1898.

TABLE IX. *Diphtheria and Scarlet Fever.—Relative Case-Mortality* (*Fatality*) *in each month of the year, based on the experience of* 1891–98.

(The average fatality for the entire period = 100.)

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Diphtheria	111	108	108	105	99	93	92	98	93	95	100	109
Scarlet Fever	130	120	130	117	107	102	90	102	76	74	96	116

The fatality rates were obtained by applying to the number of cases notified in each month the deaths of a month beginning and ending a week later, in order that so far as practicable the deaths might relate to the same patients as the cases. It will be noted that the fatality both of scarlet fever and diphtheria is greatest at the beginning of the year, falling till September or October, and then rising again. The minimum fatality of diphtheria appears to occur two or three months earlier than

TABLE X. *Case-Mortality (Fatality) of Diphtheria and Scarlet Fever among patients in the Hospitals of the Metropolitan Asylums Board in the periods* 1888–94 *and* 1904.

	All Ages	0—	1—	2—	3—	4—	5—	10—	15—	20—	25—	30—	35—	40 and upwards
Diphtheria Admissions Fatality 1888–94[1] …	30·3	61·8	63·1	55·1	48·3	39·0	28·1	10·6	4·3	4·6	5·4	4·9	4·5	17·4
Diphtheria Admissions Fatality 1904[2] …	10·0	31·3	22·3	13·0	12·7	10·6	8·2	4·1	2·6	—	—	—	3·1	—
Percentage reduction in Fatality	67	49	65	76	73	71	71	61	39	—	—	—	31	—
Scarlet Fever Admissions Fatality 1889–94[1] …	7·0	29·6	26·7	20·7	14·6	10·3	4·2	1·7	1·8	1·8	1·9	3·0	3·0	4·5
Scarlet Fever Admissions Fatality 1904[2] …	3·3	12·3	12·4	8·5	5·0	2·9	1·9	1·4	0·6	1·0	3·8	1·2	—	—
Percentage reduction in Fatality	52	58	53	59	65	71	55	18	67	63	*+100	60	—	—

---

[1] From Sir S. Murphy's *Annual Report for* 1898.

[2] From the *Annual Report of the M. A. Board for* 1904.

that of scarlet fever. In his report for 1895 Sir S. Murphy corrected similar rates for differences in age and sex distribution and found that these did not suffice to account for the variation in fatality shown in the tables.

Natural Variations in Fatality of Diphtheria.—We have already stated that the fatality of attacks of diphtheria has been lowered by the administration of antitoxin. It has also been noted that the increased use of bacteriological diagnosis leads to more complete notification of cases and a consequent lowering of the fatality of attack which is only apparent. It is difficult to decide whether apart from the operation of these two factors diphtheria has during recent years become a disease having a lower fatality. In Table X p. 79 the fatality of diphtheria at different ages is compared in 1888–94, a prae-antitoxin period, with that of 1904 among patients admitted to the hospitals of the Metropolitan Asylums Board. The corresponding facts for scarlet fever have been added for comparison.

All the percentage changes shown in Table X are declines except the one marked plus. Comparing the declines in fatality shown by scarlet fever and diphtheria respectively it is evident that they closely resemble each other, and that if the above were the only facts bearing on the problem one would naturally conclude that the changes in fatality of diphtheria, like those in scarlet fever, have been due to some extent at least to causes independent of therapeutics. Commenting on a like comparison in his report for 1898 (p. 31), Sir S. Murphy goes on to show that whereas the fatality among cases of scarlet fever treated in hospitals was greater than among home-treated cases it was much lower in hospital-treated cases of diphtheria of younger ages than among home-treated cases. The fatality statistics may on the whole be said to leave the problem unsettled. A few years' further experience will probably support the conclusion to which clinical experience strongly points that the administration of antitoxic serum greatly diminishes the fatality of diphtheria.

Seasonal Incidence of Diphtheria.—Diphtheria belongs, like scarlet fever and enteric fever, to the group of diseases whose maximum prevalence is in the autumn of each year. This is clearly shown by the following curve (Fig. 7) taken from the *Annual Summary of the Registrar-General for* 1890. The greatest mortality is seen to occur in London in October and December, the smallest mortality from May to August. The autumnal peak is not so marked as in the corresponding mortality curve for scarlet fever (Fig. 8), nor is the fall to a minimum in the spring so great in the case of diphtheria as in that of scarlet fever.

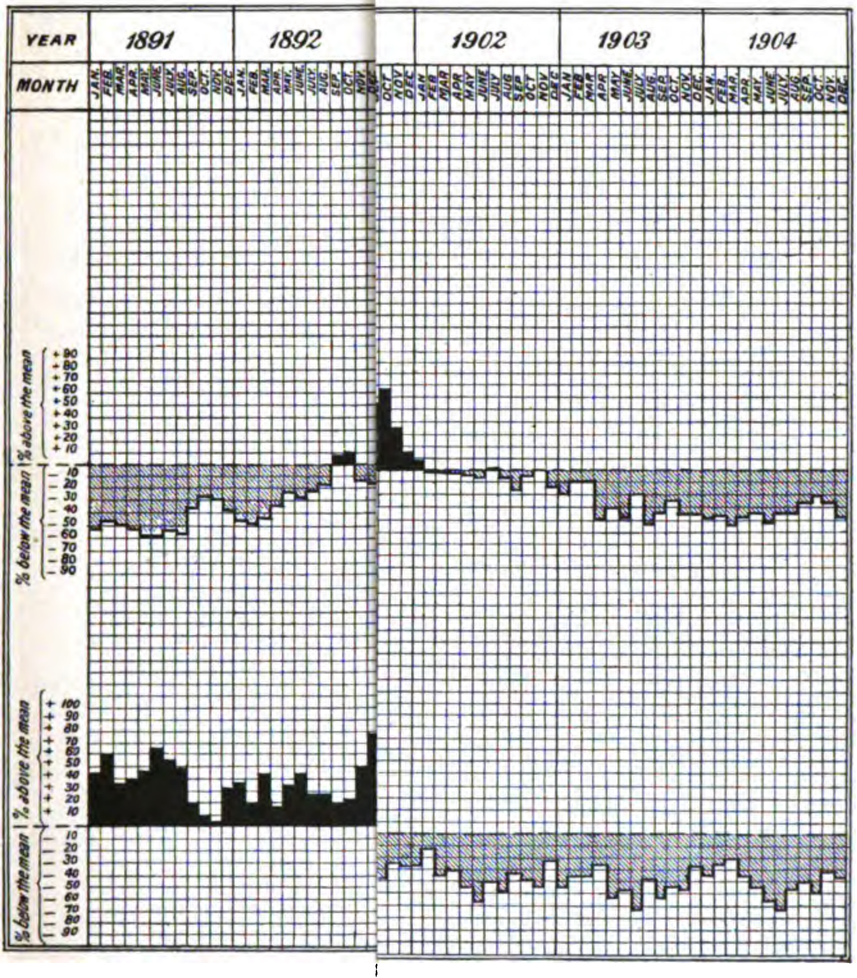
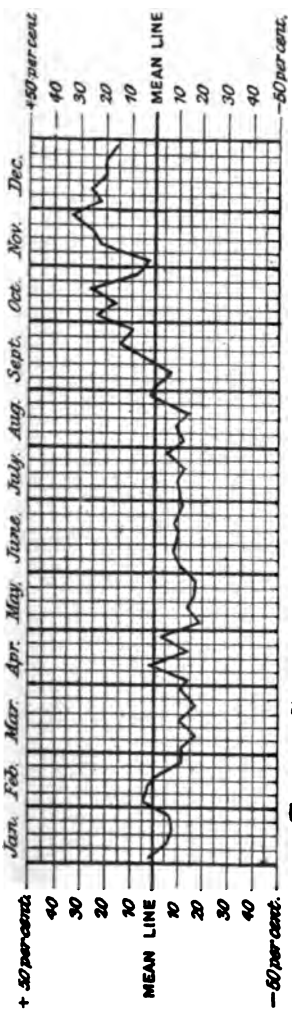


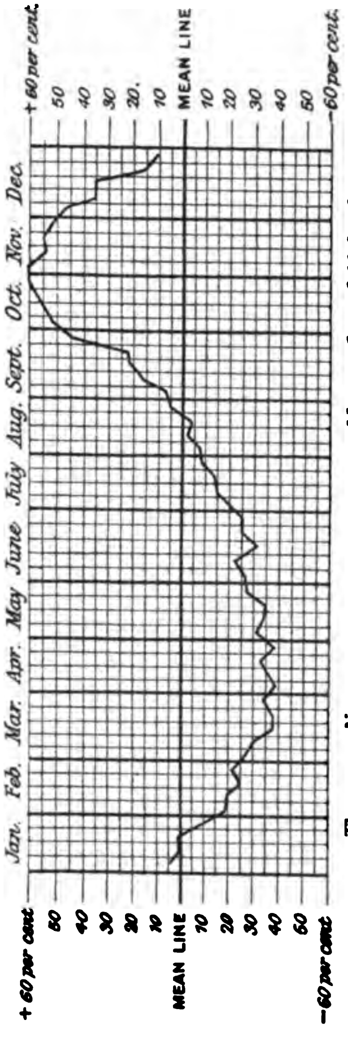
Fig. 6he same period.

The above method of statement of seasonal incidence of mortality from diphtheria represents the combined experience of 30 years. It is like a composite photograph which conceals individual characteristics while showing the type. During an epidemic the seasonal curve is obscured by the epidemic prevalence throughout the year. This is well seen in Fig. 6 in the experience of London during 1896. With a declining epidemic (as in 1903, Fig. 6) the death-rate may be higher earlier in the year than in the autumn. As a rule, however, the seasonal incidence of diphtheria conforms to the autumnal type.



The mean line represents an average weekly number of 73 deaths.

Fig. 7. Seasonal Incidence of Deaths from Diphtheria based on the experience of London in the 30 years 1861–90.



The mean line represents an average weekly number of 44 deaths.

Fig. 8. Seasonal Incidence of Deaths from Scarlet Fever based on the experience of London in the 30 years 1861–90.

SECTION III.

CHAPTER III.

THE PATHOLOGY OF DIPHTHERIA.

By F. B. MALLORY.

CONTENTS.

Introduction and Literature.

Technique.

(*a*) Eosin and Methylene Blue Stain. (*b*) Anilin Blue Stain. (*c*) Phosphotungstic Acid Haematoxylin Stain.

Varieties of Lesions.

A. Local Lesions.

Distribution of Local Lesions.

Distribution of Membrane.

Gross Characters of Membrane.

Nature of Local Lesions.

Retrograde Changes in the Epithelium.

- (*a*) Stratified squamous epithelium.
  - (1) Direct division. (2) Vacuolation. (3) Necrosis.
- (*b*) Stratified cylindrical epithelium.
- (*c*) Cubical epithelium.

Exudation.

The Histological Structure of Membrane.

The Changes in the Submucosa.

The Characters of the Membrane in different locations.

(*a*) Tonsils. (*b*) Pharynx and soft palate. (*c*) Epiglottis and larynx. (*d*) Trachea. (*e*) Tongue. (*f*) Oesophagus. (*g*) Stomach. (*h*) Duodenum. (*i*) Nose and accessory sinuses. (*j*) Conjunctiva. (*k*) Skin.

Ulceration.

The Distribution of Diphtheria Bacilli.

(*a*) In local lesions. (*b*) In the blood.

The Lungs.

(1) Bronchopneumonia. (2) Atelectasis and emphysema. (3) Interstitial tissue. (4) Necrosis and abscess. (5) Pleurisy. (6) Bacteria.

B. SYSTEMIC OR GENERAL LESIONS.
  Introduction.
  The Bone Marrow.
    (*a*) Nucleated red blood corpuscles. (*b*) Bone marrow giant cells. (*c*) Polynuclear neutrophile myelocytes. (*d*) Endothelial cells. (*e*) Eosinophile cells. (*f*) Lymphoid cells. (*g*) Plasma cells. (*h*) Myelocytes. (*i*) Premyelocytes and prelymphocytes.
  The Blood.
  The Lymphatic Tissues.
    (*a*) Lymph nodes. (*b*) Tonsils. (*c*) The thymus.
  The Spleen.
    (1) Lymph nodules. (2) The pulp. (3) The arteries.
  The Gastro-intestinal Tract.
  The Heart.
    (1) Fatty degeneration. (2) Interstitial myocarditis. (3) Mural thrombi.
  The Kidneys.
    (1) Degeneration changes. (2) Acute interstitial changes. (3) Acute glomerulonephritis.
  The Liver.
    (1) Degeneration of liver cells. (2) Necroses of the liver, (*a*) central necroses, (*b*) focal necroses.
  The Pancreas, Salivary Glands, Adrenals, Pituitary Body, Thyroid Gland, and Testicle.
  The Nervous System.
  The Skeletal Muscles.

C. SECONDARY LESIONS.
    (*a*) Accessory sinuses. (*b*) The middle ear. (*c*) Acute abscesses. (*d*) General septicaemia. (*e*) Acute endocarditis. (*f*) Acute pericarditis. (*g*) Acute pleurisy. (*h*) Acute peritonitis. (*i*) Acute suppurative mediastinitis. (*j*) Other diseases.

D. REPAIR, CAUSES OF DEATH, SEQUELAE, INCUBATION.

E. EXPERIMENTAL DIPHTHERIA.

INTRODUCTION.

The following account of the pathology of diphtheria is based on the study of 251 cases of the disease, which have come to autopsy in the Contagious Department of the Boston City Hospital, between Jan. 1, 1896, and June 30, 1905. Of these cases the first 220 were reported in 1901 in a monograph by Councilman, Mallory, and Pearce[1]. In the preparation of the following account extensive use is made of this monograph.

---

[1] *A Study of the Bacteriology and Pathology of Two Hundred and Twenty Fatal Cases of Diphtheria.* Monograph, 16 plates, 1901; also *Journ. Boston Soc. Med. Sci.* 1901, v. 137—319.

LITERATURE.

Since the following description of the lesions of diphtheria is based directly on the study of a very large number of cases covering every variety of lesion, it has not been deemed necessary to furnish a list of authorities under each heading or to give the views of each in regard to the process under discussion. For such summaries and for criticism of the views advanced the reader is referred to the monograph just mentioned. For the benefit, however, of those who may wish to consult these authorities in order to study the historical development of the present views in regard to the lesions of diphtheria, a chronological list of writers is given on p. 121.

TECHNIQUE.

In all cases the tissues were fixed in Zenker's fluid, but occasionally for certain definite purposes formaldehyde, alcohol, and other fixatives were also employed. After fixation in Zenker's fluid, paraffin sections of the tissues were stained with eosin and methylene blue by the following method, which has proved the most satisfactory routine stain for paraffin sections yet devised.

A. Eosin and Methylene Blue Stain for Paraffin Sections.

1. Fix in Zenker's fluid for 24 hours; wash in running water for 24 hours, and preserve in 80 per cent. alcohol.
2. Stain paraffin sections in a 5 per cent. aqueous solution of eosin (yellowish, soluble in water, Grübler) for thirty minutes. Sometimes it is advisable while staining to heat them in the paraffin bath for fifteen to twenty minutes.
3. Wash in water.
4. Stain for ten to twenty minutes in the following solution diluted with water in the proportion of one volume of the stock solution to four or five volumes of water:

Methylene blue (Grübler)	1 grm.
Carbonate of potassium	1 „
Water	100 c.c.

5. Wash in water.
6. Differentiate in 95 per cent. alcohol until the eosin colour returns in the section and the nuclei are sharp. Keep the section

constantly in motion, in order to maintain an even differentiation, and control the results with the low power of the microscope.

7. Treat with absolute alcohol, followed by xylol and xylol balsam.

Two other stains were also much employed and often proved useful, the anilin blue connective tissue stain for the study of connective tissue fibrils and the formation of the hyaline membrane, and the phosphotungstic acid haematoxylin stain for the demonstration particularly of fibrin.

B. Anilin Blue Connective Tissue Stain.

1. Fix in Zenker's solution and embed in paraffin or celloidin.

2. Transfer sections from water to a one-tenth per cent. aqueous solution of acid fuchsin for five minutes.

3. Transfer sections directly (without washing) to the following stain for two to ten minutes:

Anilin blue soluble in water (Grübler)	0·5 grm.
Orange (Grübler)	2·0 „
One per cent. aqueous solution of phosphomolybdic acid	100·0 c.c.

4. Differentiate and dehydrate quickly (rarely over one to two minutes) in two or three changes of 95 per cent. alcohol followed by absolute alcohol for paraffin sections.

5. Clear in xylol (filter paper blotting method for celloidin sections) and mount in xylol balsam.

Fibrin and neuroglia, myoglia, and fibroglia fibrils are red, intercellular connective tissue fibrils blue, elastic fibrils yellow or pale pink.

C. Phosphotungstic Acid Haematoxylin Stain.

1. Fix in Zenker's solution and embed in paraffin or celloidin.

2. Transfer sections from water to a one-fourth per cent. aqueous solution of permanganate of potassium for two to five minutes (rarely ten to twenty minutes; especially for neuroglia fibrils).

3. Wash in water.

4. Place in a 5 per cent. aqueous solution of oxalic acid for five to ten minutes (rarely one to two hours).

5. Wash thoroughly in several changes of water.

6. Stain in phosphotungstic acid haematoxylin for twenty-four to forty-eight hours.

7. Wash quickly in water.
8. Dehydrate in 95 per cent. alcohol followed by absolute alcohol for paraffin sections.
9. Clear in xylol (filter paper blotting method for celloidin sections) and mount in xylol balsam.

Fibrin and fibroglia, myoglia, and neuroglia fibrils are blue; intercellular connective tissue fibrils pale reddish brown or colourless; the coarse elastic fibrils pale blue. If, after step 6, the sections are placed in a strong alcoholic solution (ten to twenty per cent.) of chloride of iron for one to several minutes, followed by thorough washing in water, the intercellular connective tissue fibrils are completely decolorised and the blue-stained fibrils take a somewhat deeper tint[1].

VARIETIES OF LESIONS.

Two varieties of lesions occur in uncomplicated diphtheria, (1) the local, and (2) the general or systemic. The *local lesions* are due to the strong toxic substances which are secreted by the diphtheria bacilli and act on the tissues in the immediate vicinity of the bacilli. These lesions are usually characterised by the formation of a membrane. The *systemic* or *general lesions* are due to diphtheria toxin which is absorbed and passes along the lymph and blood-vessels to act in a dilute form on the tissues. These two kinds of lesions are usually complicated in cases

---

[1] *Phosphotungstic Acid Haematoxylin.*—When this formula was first published the phosphotungstic acid manufactured by Merck was not pure. It contained a trace of phosphomolybdic acid, and also some oxidising agent which ripened haematoxylin at once. The acid obtained since then sometimes oxidises haematoxylin and sometimes does not. It is a simple matter, however, to ripen the solution at once.

Haematoxylin	0·1 gram.
Water	80 c.c.
10 per cent. aqueous solution of phosphotungstic acid (Merck) ...	20 c.c.

Dissolve the haematoxylin in a little water by the aid of heat, and add it when cool to the rest of the solution. If the solution does not stain, it may be ripened by the addition of either of the following oxidising reagents: peroxide of hydrogen (U.S.P.), ·2 cubic centimetre or one-fourth per cent. aqueous solution of permanganate of potassium, ten cubic centimetres.

The best results are obtained by using haematein ammonium instead of haematoxylin, and allowing the solution to ripen spontaneously for several months. Solutions a year or more old stain with great intensity.

If haematein ammonium is used instead of haematoxylin and it is desired to ripen the solution at once, use half the quantity of either of the oxidising reagents mentioned above.

which come to autopsy by a third variety of lesion due to secondary infections with various other micro-organisms. This kind of lesion will be spoken of as *secondary*. On account of these secondary infections it is impossible in the following account to determine exactly what part of the lesions present in certain organs, such as the tonsils, the lungs, and the cervical lymph nodes, is due to the diphtheria bacillus, and what part may be due to absorption of the toxins secreted by the *Streptococcus pyogenes* and other micro-organisms.

A. LOCAL LESIONS.

Distribution of the Local Lesions.

The local lesions begin most frequently in the pharynx (tonsils); the larynx; the nares; or the lungs; rarely in the conjunctivae. From these primary foci the pathological process often extends; from the tonsils to the uvula, the pillars of the fauces, and the posterior wall of the pharynx; from the larynx to the trachea and lungs; or from the nares to the accessory sinuses of the nose. More rarely it extends from the primary foci to the oesophagus, tongue, or skin round the nose and mouth; or to the middle and external ear through the Eustachian tube and a perforated membrana tympani. Still more rarely diphtheria bacilli which have been swallowed cause lesions in the stomach, or are conveyed to the genitals (vulva, vagina, penis) by the fingers and give rise to membrane formation there.

The distribution, extent, and intensity of the local lesions vary greatly in different cases. Statistics in regard to them should be based as far as possible on clinical observations rather than on post-mortem findings which represent conditions in fatal cases only, and are therefore likely to give rise to a false impression. On the other hand it is only by means of post-mortem examinations that the full extent of the distribution of local lesions can be determined in certain cases (stomach, accessory sinuses of nose, etc.).

Distribution of the Membrane.

In this series of 251 cases a definite membrane, which varied in extent and distribution, occurred in 148. It was found on the larynx in 86, on the tonsils in 74, in the trachea in 73, on the epiglottis in 67, in the bronchi in 44, on the mucous membrane of nares in 43, on the soft palate, including the uvula, in 15, in the oesophagus in 12, on the tongue in 9, in the stomach in 5, in the duodenum in 1, on the vagina in 2, on the vulva in 1, on the skin of the ear in 1, and on the conjunctiva in 1. In a number of the cases the membrane was small in amount and limited to a single situation. It was confined to the tonsils alone in 9 cases, to the larynx in 5, to the trachea in 3, to the pharynx in 2, to the epiglottis in 2, to the soft palate in 1, to the oesophagus in 1, and to the mucous membrane of the nares in 1. In all other cases several structures were involved.

PLATE I.

Fig. 1. Direct division of nuclei of epithelial cells. × 1000.

Fig. 2. Fibrinous membrane in the trachea showing flattening of the spaces in the reticulum. Between the denser portion of the fibrinous reticulum and the basement membrane the spaces in the reticulum are larger and contain numerous cellular elements, chiefly endothelial cells. In the upper layer of the fibrinous membrane are many polynuclear leucocytes. × 500.

I am indebted for the microphotographs reproduced in Plates I, II, III, IV and V (which were taken with a Zeiss apparatus of the latest design lately purchased by the Boston City Hospital) to Dr S. Burt Wolbach, Assistant in Pathology at the Harvard Medical School, and to Dr Samuel T. Orton, Second Assistant in Pathology at the Boston City Hospital.

The Gross Characteristics of the Membrane.

The membranes vary greatly in appearance. The membrane may appear white, dirty white, brownish, grayish brown, or almost black in colour. It may form a thin pellicle which is easily removed, leaving a smooth surface, or a thick, tough layer which is stripped off with difficulty from the ragged, injected tissue beneath. It may be soft and granular, breaking into small fragments, or tough and elastic, and removable in large patches. The membrane is always more easily removable from the trachea than from any other part. On opening the trachea the membrane often appears as a loose wrinkled mass lying in the lumen, but at other times it is closely applied to the wall. Even when thick and adherent its removal rarely leaves a loss of substance extending into the subepithelial tissue.

The tissue beneath and adjoining the membrane is always intensely injected and often haemorrhagic; the injection is usually general but occasionally may be more evident in foci. The injection is always well seen on microscopic examination, but macroscopically may be obscured by superficial necrosis.

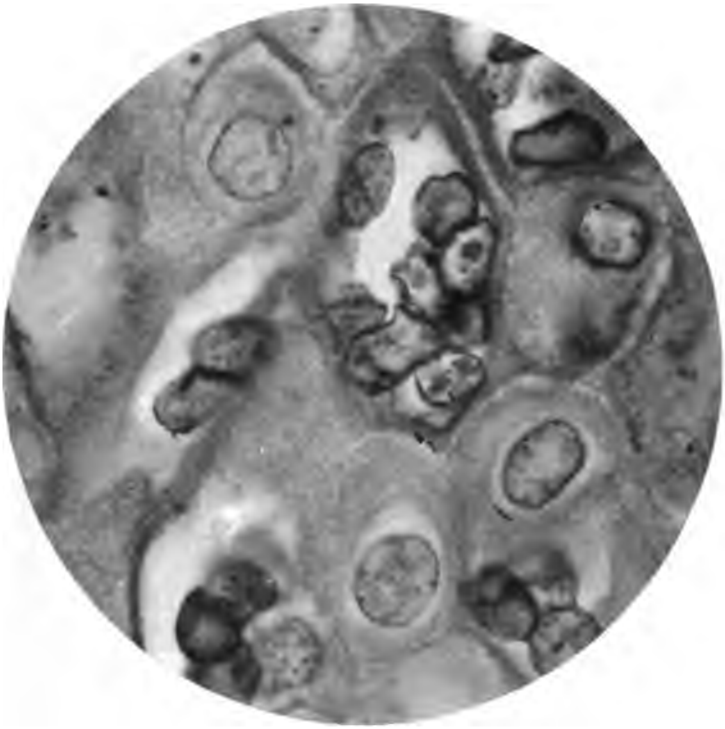


Fig. 1.

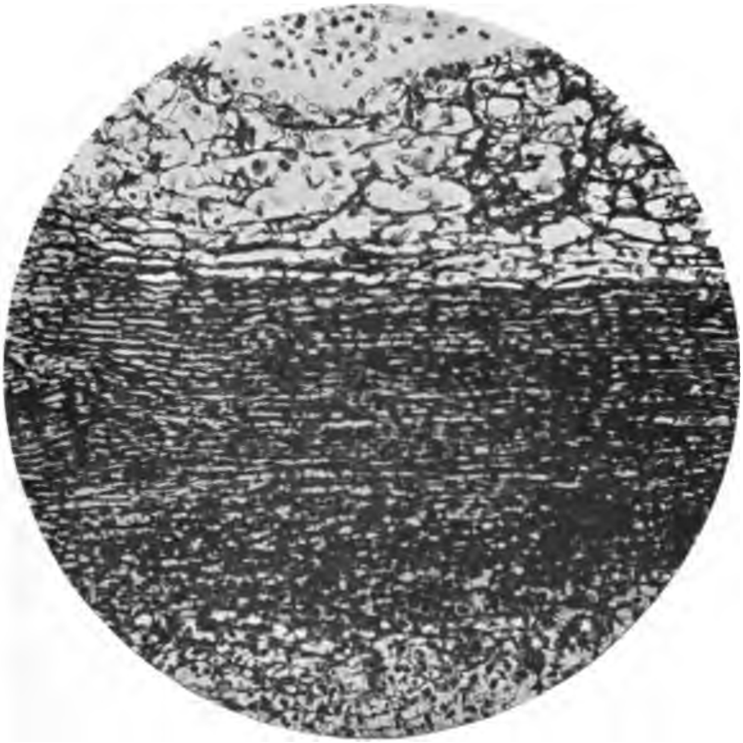


Fig. 2.

Nature of the Local Lesions.

The local lesions show in all situations essentially the same pathological processes, namely, degenerative changes in the epithelial cells and in the underlying tissues, combined with an abundant fibrinous exudation from the blood-vessels. As a result of these degenerative and exudative processes, separately or combined, a membrane is formed on the epithelial surface affected. This membrane formation is so striking and characteristic a feature that the disease has been named from it.

The primary lesion can be best understood by studying it biologically, that is to say by starting with the earliest recognisable changes in the epithelium and tracing the process through to the development of the severest lesions. In doing this it is advisable to consider the epithelium separately from the submucosa, and further to take up in each case the degenerative changes and then the inflammatory exudation.

Retrograde Changes in the Epithelium.

Under this heading it is necessary to include the stratified squamous epithelium covering the pharynx, tonsils, uvula, epiglottis, and larynx; the stratified ciliated cylindrical epithelium lining the ventricles and lower end of the larynx, the trachea, and the bronchi in which it gradually thins out to a single layer; and the cylindrical and cubical epithelium lining the ducts and acini of the numerous mucous glands opening into the respiratory tract.

In general it may be said that the degenerative changes in these various epithelial cells vary greatly in extent and character. They are most easily found and studied in those places which are covered with squamous epithelium because the cells do not readily desquamate, and in the mucous glands where the cells necessarily remain *in situ.*

The most noticeable degenerative changes are multiplication of the nuclei by direct division, vacuolation of the cytoplasm, and necrosis of the cells with disintegration of the cytoplasm and fragmentation of the nuclei, or with hyaline transformation. The extent and distribution of these various forms of degeneration can be best appreciated by considering separately the different situations in which they occur.

(*a*) *Stratified squamous epithelium covering the tonsils, uvula, pharynx, epiglottis, larynx, and tongue.*

It is usually easy to study the degenerative processes in all their stages in these situations since all degrees of the diphtheritic lesion are often present in a single case; this is due to the fact that the lesion usually begins in separate foci and spreads; between the affected areas the tissues may be almost, or quite, normal.

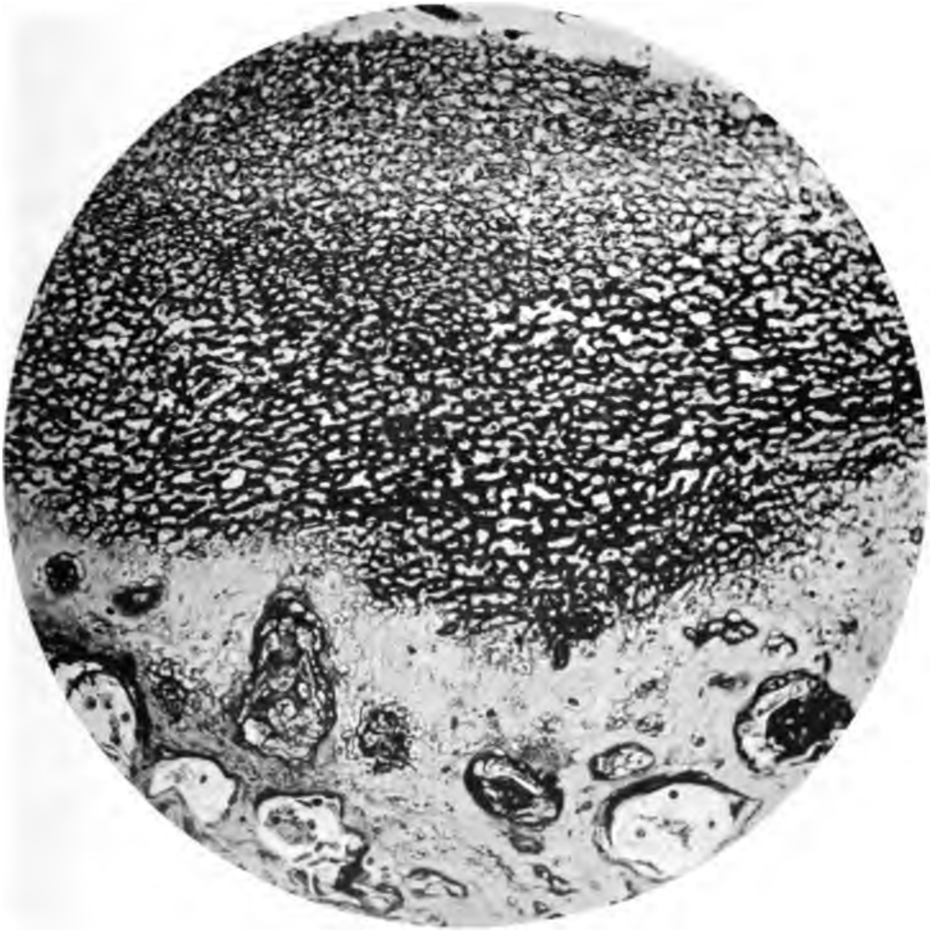
(1) *Direct division.* Large cells, each with a large lobulated nucleus or with two to ten large nuclei produced by direct division, are not numerous as a rule but sometimes occur in great numbers (Pl. I, fig. 1, p. 88). They are found chiefly in the upper layers of the epithelial cells.

(2) *Vacuolation.* In the same situation are often found large vacuolated cells which may contain varying numbers of bodies of various sizes, which are often reticulated, and are evidently derived from the breaking down of the cytoplasm. They may also contain threads of fibrin.

(3) *Necrosis.* Necrosis may affect single epithelial cells or groups of cells. In some cases the nuclei undergo fragmentation, but in other cases the first evidence of necrosis is a solidification and hyaline transformation of the cytoplasm. The nucleus often remains for some time normal in its appearance and staining reactions; later as a rule it disappears, leaving a space in the cell. The necrosis in some cases extends through the whole layer of stratified epithelium to the underlying submucosa. Occasionally necrosis and vacuolation may occur together in the same cells. A rare phenomenon is the formation of one to several irregular, hyaline, acid-staining bodies within the nucleus. Sections stained in Scharlach R show marked fatty changes in the epithelial cells in situations in which degenerative changes are taking place.

(*b*) *Stratified cylindrical epithelium lining the ventricles and lower end of the larynx, the trachea, and the bronchi.*

In these situations the lesion spreads very uniformly, but is usually most marked in the larynx and upper part of the trachea. The epithelium desquamates very quickly and often only the lowest layer of it is left intact, so that it is not easy to study the degenerative changes. Here the epithelial cells show fatty changes and may contain hyaline droplets of various sizes in the cytoplasm, and sometimes nuclear



Hyaline membrane in pharynx. The reticulum is coarse, the spaces small and rounded; they contain few cells. In the submucosa the walls of the blood and lymph vessels show marked hyaline fibrinoid change. ×500.

masses due to direct division. Polynuclear leucocytes are often present in considerable numbers between the epithelial cells. There seems to be little or no tendency for the epithelial cells of the stratified cylindrical type to undergo necrosis with hyaline change.

(c) *Cubical epithelium of the ducts and acini of mucous glands.*

Lobulation and direct division of the nuclei occur to some extent, and swelling and vacuolation of the cytoplasm are sometimes found, but the most common and prominent change is necrosis. This, however, almost invariably begins first in endothelial cells which have invaded the acini from without and have pushed the lining epithelium away from the basement membrane. It is difficult to find these cells so as to make sure of their character before they have become necrotic; so quickly do they succumb. The first change noticed in them is the deep staining of the cytoplasm with eosin; then the nucleus stains deeply and uniformly with the nuclear dye, and later it undergoes fragmentation.

Following this necrosis of endothelial cells the epithelium undergoes degenerative changes. The cytoplasm becomes filled with innumerable small acid-staining globules of various sizes, but usually small. The cells become separated from each other. The process is often so extensive that the acini are entirely replaced by cell fragments.

Exudation.

As a result of these degenerative changes in the epithelium and accompanying them more or less closely, an inflammatory exudation, which is usually abundant, takes place. This exudation complicates the appearance presented by the degenerating epithelium. The serum may collect in the cytoplasm of degenerating epithelial cells causing vacuolation, or it may separate the epithelial cells more or less from each other, or it may accumulate between the epithelial cells just below the cornified layer of the stratified squamous epithelium and lead to the formation of microscopic vesicles. In such cases appearances similar to the small-pox vesicles may be seen. In this fluid fibrin often forms, namely, within the vacuoles in the cells, within the minute vesicles just mentioned, and between the more or less separated epithelial cells. Much of the fluid part of the exudate unquestionably escapes from the surface.

The cellular portion of the exudate consists chiefly of polynuclear leucocytes and endothelial cells (large mononuclear leucocytes). To a less extent lymphoid and plasma cells and rarely eosinophile cells are present. These leucocytes pass between the epithelial cells and often invade those which are necrotic. Many of them undergo necrosis and fragmentation under the influence of the diphtheria toxins. The endothelial cells are phagocytic and often contain numerous inclusions in their cytoplasm. As a rule the endothelial cells are found in the deeper layers of squamous epithelium, the polynuclear leucocytes in the superficial layers and on the surface. The exudation always separates the epithelial cells to some extent from each other. Red blood corpuscles are often carried into the epidermis by the exudation and occasionally they are taken up by the cytoplasm of epithelial cells, in which they undergo degenerative changes. The greatest number of red corpuscles included within epithelial cells was found in the uvula in the vicinity of the membrane on it.

In the trachea and bronchi the epithelial cells desquamate so easily and quickly that except at the very beginning of the process the exudation has little or no opportunity to collect in the epithelium.

The exudation in diphtheria is rich in fibrin factors, and fibrin is usually formed quickly and abundantly. The fibrin threads are laid down not only on the surface of the epithelium, but also between the separated epithelial cells. It is this deposition of fibrin on the surface of the epithelium and between the epithelial cells which produces the membrane so characteristic of diphtheria. It is formed as a result of a primary injury to the epithelium.

PLATE III.

Fig. 1. Hyaline membrane in the stage of transformation from hyaline degenerated epithelial cells. × 1000.

Fig. 2. Central necrosis of the liver. Between the necrotic liver cells and the walls of the compressed sinusoids is a haemorrhagic exudation containing polynuclear leucocytes and endothelial cells some of which are invading the necrotic liver cells. × 1000.

The Histological Structure of the Membrane.

The membrane in the beginning, therefore, always consists of fibrin. It forms a reticulum, the so-called *fibrinous reticulum*, or *membrane* (Pl. I, fig. 1, Pl. II), which is very characteristic. The spaces in the reticulum vary considerably in size, but are usually larger than in the hyaline membrane described below. They are generally flattened so that their long axes are parallel with the surface on which the membrane rests.

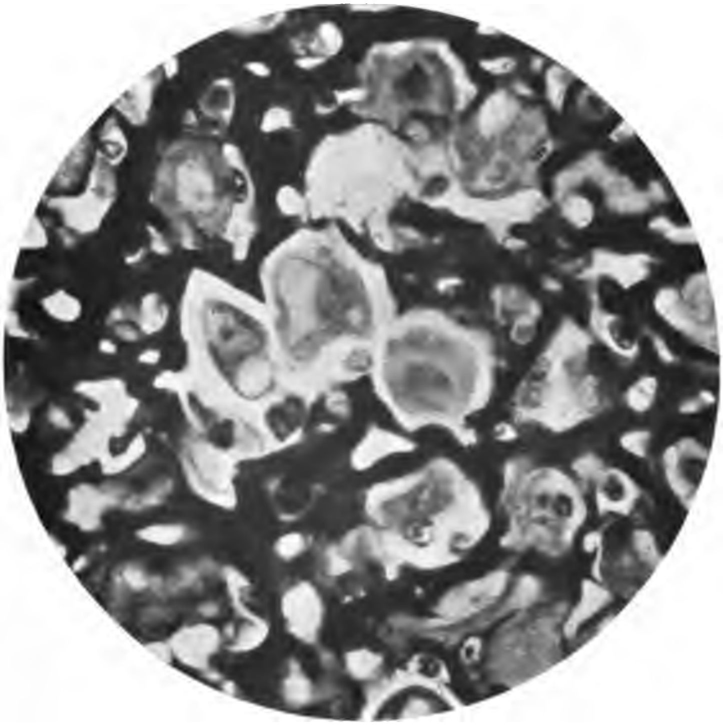


Fig. 1.

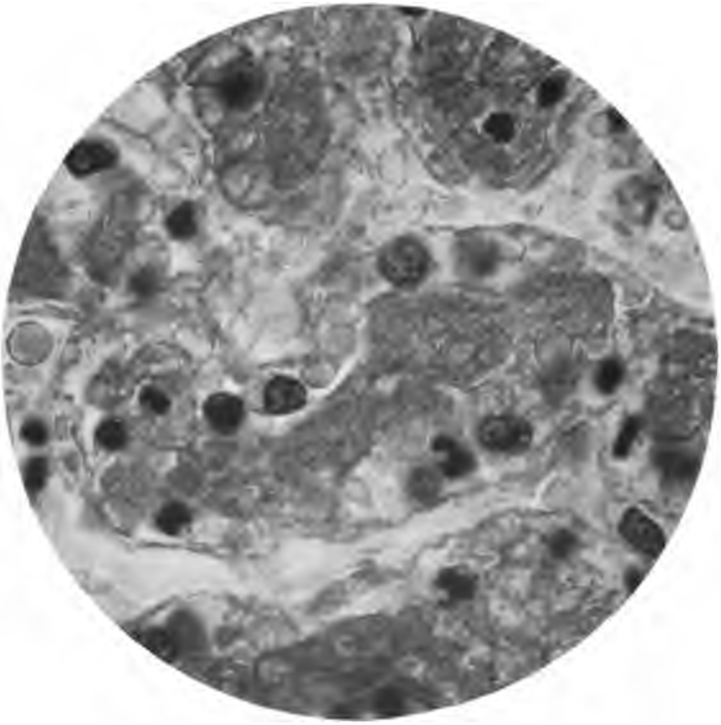


Fig. 2.

Frequently the reticulum appears arranged in whorls starting from a centre. The thickness of the individual fibrin threads varies: in freshly-formed fibrin they are usually rather delicate and straight, but as the membrane ages the fibrin-threads become thicker and their outlines curved. Numerous polynuclear leucocytes, red blood corpuscles, and endothelial and epithelial cells, the latter being often hyaline, are usually included in the meshes of the reticulum.

Where the fibrin forms around hyaline degenerated epithelial cells the two elements are at first perfectly distinct in outline and in staining reactions. For instance, with the anilin blue connective tissue stain, the fibrin is intensely red, while the hyaline cells appear bluish green. Gradually, however, the cells and the fibrin become more or less perfectly fused together as the result of being bathed in fibrin-forming materials, so that in a short while the two elements are indistinguishable. The reticulum formed in this manner usually stains more or less perfectly like fibrin. It has a very characteristic appearance and is known as the *hyaline reticulum* or *membrane* (Pl. III, fig. 1). The beams of which it is composed are thick and dense, and have been compared to young osteoid tissue. The spaces within it are usually small; they may be angular or round, but are sometimes flattened by pressure or stretching. Some represent spaces left by the disappearance of nuclei, others vacuoles formed in the cytoplasm, and others spaces between the cells not filled in by fibrin. This is shown by the frequent presence in these membranes of epithelial cells in all stages of transformation to a hyaline reticulum. On the other hand no evidence can be found to support the view that the epithelium *in situ* can be transformed directly into a hyaline reticulum without fibrin playing any part in the process.

It is possible that endothelial cells and even polynuclear leucocytes may undergo hyaline change and fuse with fibrin to form a hyaline reticulum. It is even more possible that a fibrin reticulum may in time by simple thickening due to the deposition of fresh fibrin on its surface be transformed into a hyaline reticulum. It is difficult, however, to trace all the stages of such a transformation so as to be sure of it. The fact that the hyaline membranes which are formed on surfaces occur chiefly, if not exclusively, on those covered with stratified squamous epithelium is a strong argument in favour of the view that epithelial cells which normally undergo a hyaline process (cornification) are necessary for their formation. In the subcutaneous connective tissue, as will be shown later, necrotic connective tissue may play a similar *rôle.*

The hyaline membrane is never found to the exclusion of the fibrinous reticulum but always in varying combination with it. Its upper surface is rarely well defined as the reticulum becomes swollen and more or less disintegrated.

Fibrin forming on surfaces covered with stratified squamous epithelium is closely bound down by fibrin strands running in every direction between the epithelial cells still attached to the surface. The resulting membrane, whether hyaline or not, cannot, therefore, be readily stripped off. It remains *in situ* and is bathed in fresh fibrin factors which thicken the reticulum already formed.

On surfaces covered with stratified cylindrical epithelium, such as the trachea, the epithelial cells are fewer in number and desquamate quickly. Beneath them is a thick hyaline basement membrane to which the fibrin does not easily become attached. As a result the older part of the fibrinous membrane is gradually pushed off and new fibrin is deposited beneath it. Beneath the membrane the epithelium is usually absent. In places small masses of epithelium consisting of the lower layers of cells are found, and sometimes the membrane may extend over a considerable area of epithelium which is but little altered, although the upper layers of cells are always absent. Both kinds of membrane, but especially the fibrinous, often show areas which differ in character from the main mass of the membrane. Areas, for example, are found where the reticulum is broken and forced apart, and the spaces thus formed are filled by a fresh fibrinous network, of which the fibres are delicate and the spaces large and irregular, and filled with large numbers of leucocytes. The membrane proper may even be elevated from the surface by such a formation. It is obvious that after the membrane was formed a fresh exudation took place, forcing apart its meshes or elevating it bodily. Sometimes the membrane contains large pockets filled with polymorphonuclear leucocytes and fresh fibrin.

The Changes in the Submucosa.

The blood-vessels are engorged and the lymph vessels dilated, and the endothelial cells lining them prominent. There is general oedema of the tissues with much diffuse formation of fibrin in the fluid. Much of the finer fibrin found in sections is probably formed after death. It occurs in small islands, and as an extensive meshwork extending throughout the oedematous areas.

The cellular exudation in the submucosa varies, though as a rule polynuclear leucocytes are abundant. On the other hand endothelial cells are common and polynuclear eosinophiles are often found. Plasma cells are usually present and may occur in great abundance, especially round the mucous glands. Diapedesis of red blood corpuscles is frequent and there may be slight or extensive haemorrhages.

Often the deeper lymphatics are dilated and contain numerous polynuclear leucocytes and endothelial cells in addition to varying amounts of serum and fibrin.

Sometimes the membrane formation extends into the submucosa. This condition happens when the epithelial cells are all necrotic and the destructive process extends to the underlying connective tissue. Then the connective tissue fibrils are bathed in fibrin factors and become coated with a layer of fibrin. At first the characteristic staining reactions of each are preserved, but soon they become more or less perfectly fused together and form a hyaline reticulum, which structurally may be indistinguishable from that formed on an epithelial surface. This so-called *hyaline fibrinoid degeneration* of the connective tissue is more marked in the epiglottis than elsewhere, but the same process may take place, though rarely, in the trachea after destruction of the dense hyaline basement membrane on which the epithelium rests.

The smooth muscle fibres of the blood-vessels frequently undergo a somewhat similar hyaline fibrinoid change (Pl. II), although no distinct hyaline reticulum is formed. These fibres seem very susceptible to the action of the diphtheria toxins and readily undergo necrosis. Fibrin is deposited around and fuses with them to form dense masses which stain deeply with the fibrin stain. The process of necrosis and hyaline change may extend to the tissues around the vessels and also to the contents of the vessels. This latter change is especially frequent in the small vessels near the surface, many of which may become occluded by hyaline masses within them.

In the pharynx degenerative changes are frequent in the striated muscle fibres. The striations disappear and the fibres become converted into swollen, homogeneous, refractive masses which may be invaded by polynuclear leucocytes.

The Characters of the Membrane in different Locations.

(*a*) *Tonsils.*—Membranes on the tonsils show great variations in structure. A fibrinous membrane is often continuous over the entire surface of the tonsil and extends into every crypt. On the other hand a hyaline membrane is never found extending from the surface into the crypts, although occasionally small masses of hyaline reticulum are found in them.

A hyaline membrane may cover the entire surface of the tonsil, or only fragments of it may be found enclosed in fibrin, or it may form the upper part only of the entire membrane, being separated from the tissue beneath by fibrin.

(*b*) *Pharynx and Soft Palate.*—The membrane in these situations is in general similar to that over the tonsils; the fibrinous form predominates (Pl. II). Small quantities of mucus are occasionally present in the membrane.

(*c*) *Epiglottis and Larynx.*—Membranes on the epiglottis are usually extensive, covering both sides as dense grayish or black layers. Hyaline membrane is not so frequent as on the tonsils. The membrane in the larynx does not differ from that covering the epiglottis.

(*d*) *Trachea.*—The changes which occur in the trachea in connection with membrane formation are in many respects different from those which occur in other tissues. In general the membrane is distinctly fibrinous (Pl. I, fig. 2). Only in one instance in an early and very acute case in a child one year old was the membrane hyaline, and this condition was only present in places. It differed from the hyaline membranes found on the tonsils in the very small size of the spaces in the reticulum. In fibrinous membranes the reticulum is much closer than in similar membranes found elsewhere, the meshes being almost always flattened and the long diameter of the spaces parallel to the surface. In some cases the reticulum, especially in its upper portion, is filled with more or less degenerated cells, chiefly polynuclear leucocytes.

Between the older, denser part of the fibrinous reticulum and the membrana propria a layer composed of fresh fibrin showing a coarse meshwork is usually found. This layer is probably due to exudation which is constantly taking place and which forces the formed membrana away from the surface to which it is still connected in places by strands of dense fibrin which run down perpendicularly to the membrana propria. In this layer of fresh fibrin are numbers of phagocytic endothelial cells and many eosinophile cells. Along the membrana propria cells are often arranged in a row which by their general character and their cuticular surface can be recognised as epithelial.

The membrane usually extends over the mouths of the mucous glands without passing into them. In some cases, on the other hand, it extends directly into the ducts of the mucous glands, the epithelial lining of which is necrotic. The membrana propria in most places is intact and forms a sharp line of separation from the membrane above and the tissue beneath. Occasionally a leucocyte may be found passing through it along an irregular line.

Here and there along the surface just below the membrana propria there are small areas representing hyaline fibrinoid metamorphosis of the connective tissue. Rarely the membrana propria is destroyed and the fibrinous membrane is directly connected with the hyaline fibrinoid connective tissue.

(*e*) *Tongue.*—In nine cases of this series there was formation of membrane on the tongue due to extension from the tonsils. In most cases the epithelium had entirely disappeared and the membrane was composed of fibrin without any hyaline material. The membrane was usually thicker on the apices of the papillae than elsewhere. In one case every papilla in the section was covered with a cap of fibrin and between there was no membrane.

(*f*) *Oesophagus.*—Membrane was present in the oesophagus in twelve cases and its formation could be clearly studied. Macroscopically there were longitudinal erosions covered by thin membrane and adjoining them longitudinal areas where the surface was thickened and covered with a grayish necrotic looking deposit. In the more normal tissue between these lesions the epithelial cells were swollen and vacuolated, and some were converted into refractive hyaline masses.

The edges of the erosions were sharply cut, and the epithelium adjoining them was undermined and showed a marked degree of nuclear multiplication by direct division. In places where fibrin was macroscopically evident, microscopically a fibrinous reticulum was found with here and there small areas of thicker hyaline membrane.

(*g*) *Stomach.*—Membrane formation in the stomach is rare. It occurred in varying amounts in five of the present series of cases. In one case almost the entire surface of the mucous membrane beginning at the cardiac orifice was covered with a ragged grayish-brown membrane which was thicker over the rugae and easily removed, leaving a red granular surface beneath. In the other cases the formation of membrane was not so extensive and was limited to lines on the surface of the rugae. The mucous membrane elsewhere was very hyperaemic and there were small scattered haemorrhagic points. Microscopic examination showed the membrane to be fibrinous, and in no instance was any of the dense hyaline membrane so characteristic of the pharynx found. The membrane was always attached to a surface denuded of epithelium, and the fibrin rarely extended to any depth into the tissue below. In several cases it extended from its place of origin over the epithelial surface on all sides for a considerable distance.

(h) *Duodenum.*—In one case the surface was covered with a membrane similar in its macroscopic aspects to the membrane which was found in the stomach of the same case. Microscopically there was superficial necrosis covered with a mass composed of pus cells, nuclear detritus, necrotic epithelium, and mucus, but containing no fibrin.

(i) *Nose and Accessory Sinuses.*—Membranes occurring in the nose and accessory sinuses are fibrinous and present no unusual features.

(j) *Conjunctiva.*—Many cases of diphtheritic conjunctivitis have been reported, but in this series it only occurred once and no microscopic examination of it was possible.

(k) *Skin.*—In the literature (see Index) many cases of diphtheria of the skin have been reported, for example, of the skin around the anus, of the penis, of the lips and gums, and of the female genitalia. In this series of cases one occurred in which a distinct membrame was formed on the external ear following diphtheritic otitis media with perforation of the membrana tympani. Microscopically the membrane was fibrinous in character and enclosed in its meshes polynuclear leucocytes and in places hyaline degenerated epithelial cells.

Ulceration.

Ulceration of the tissues, which are the seat of the local lesion, occurs in a small proportion of cases. In this series distinct ulceration was found in twelve cases, five times involving the tonsils, three times the larynx, twice the trachea, once the pharynx, and once the epiglottis. All of these were severe cases in which intubation was performed. In the tonsils the ulcers, which were foul and ragged and bordered by necrotic tissue, were produced by extensive necrosis extending from the membrane into the tissue. Those in the pharynx and epiglottis were of the same character as in the tonsils, though not so extensive, while those in the larynx and trachea were clearly due to the influence of the intubation tube. The tracheal ulcerations were the least marked.

The Distribution of the Diphtheria Bacilli.

(a) *In Local Lesions.*—Diphtheria bacilli can almost always be demonstrated microscopically in early cases when a definite membrane is present. They occur in clumps of varying size chiefly on the surface of the membrane, but also in necrotic tissue. They are not found in living tissue, or on the surface of normal epithelium, or even in those places where there are degenerative changes in the epithelium which can be regarded as the primary lesion of the disease. It seems probable that the beginning of the lesion is due to the toxic action of bacilli growing in the fluids of the mouth or throat. When necrosis is once produced the necrotic tissue and the membrane deposited on its surface form a suitable culture medium. In the accessory sinuses of the nose and in the middle ear the diphtheria bacilli so frequently present often produce only a mucoid or purulent secretion.

(*b*) *In the Blood.*—In a certain number of cases the cervical lymph nodes become infected with diphtheria bacilli from the local lesions in the throat, and the bronchial nodes from lesions in the lungs without the bacilli reaching the circulation. In many fatal cases, however, the bacilli get into the blood and can be obtained in cultures from various organs. The order of relative frequency is as follows, liver, kidneys, spleen, heart's blood, and very rarely the brain. The highest percentage of septicaemia recorded is 50, but in this series general septicaemia was only found in about 20 per cent.

The Lungs.

The lesions of the lungs must be considered in connection with the local lesions of diphtheria because they are often due entirely or in part to the immediate presence of the diphtheria bacillus. At the same time the slight lesions of systemic origin which appear in the lungs and the more extensive secondary lesions due to invasion by other micro-organisms must be described.

In general it may be said that the lungs in diphtheria are voluminous, contracting but little on opening the thorax. In most cases there is considerable, and occasionally extreme, injection of the vessels. Ecchymoses frequently occur in the pleura, measuring 1 to 10 mm. in diameter, and are most numerous over the lower and posterior surfaces of the lungs.

1. *Bronchopneumonia.*—The most common lesion is bronchopneumonia. The term implies both the manner in which infection takes place, and the relation of the foci to the bronchi. The areas of solidification vary considerably in size. In many cases they are sharply limited to lobules, in others they are much smaller, and in some they involve a number of lobules. Cases of extensive confluent

bronchopneumonia sometimes occur, but not of true acute lobar pneumonia.

The character of the solidification varies. In some cases the foci are so sharply circumscribed as to resemble tuberculous areas, but generally they are more diffuse and fade gradually into the surrounding tissue. They are usually of a reddish colour, but in some cases they are grayish red or even gray. The larger areas of solidification are not homogeneous, but contain slightly elevated centres which are firmer than the surrounding tissue, making it evident that these areas are formed by the confluence of adjoining smaller areas.

Such areas of bronchopneumonia were found in 60 % of the cases here studied. In the majority of them the posterior portion of the lungs was affected, and especially the lower lobes. The occurrence of the discrete or of the confluent form does not appear to bear any relation to the variety of micro-organism present, nor does the presence of membrane in the bronchi appear to influence the extent of the solidification. There is, however, a very definite relation between the presence of membrane in the lower respiratory passages and the occurrence of bronchopneumonia. Thus of the cases with membrane in one or more of the lower respiratory passages (epiglottis, larynx, trachea, or bronchi) bronchopneumonia was present in 72 %, while in the remaining cases it was present in only 48 %. The fact that of the 76 cases which were intubed 80 % showed bronchopneumonia, is of still greater interest.

The tissue in the vicinity of the foci of solidification was generally oedematous, but there was little general oedema of the lung comparable to that so commonly found in adults. The oedema was distinctly related to the inflammation, and was not the general oedema due to disturbances of the circulation.

Acute inflammation of the larger bronchi usually accompanied the bronchopneumonia, but was not constant. When present the mucous membrane was reddened and covered with exudation, and usually small drops of pus could be forced from the smaller bronchi on pressing the cut surface of the lung. In 43 cases there was a fibrinous exudation, forming in the larger bronchi a distinct membrane and completely filling the smaller. The membrane was similar to that found in the trachea.

Microscopic examination shows that the process begins as an infection of the atria and extends. It may be limited to single acini, to lobules, or to groups of lobules. There is but little lateral extension of the infection through the walls of the alveoli or of the bronchi into the surrounding air-spaces.

The character of the exudation varies greatly, being fibrinous, haemorrhagic, serous, or almost entirely cellular. The cells in the exudation are partly polynuclear and large mononuclear leucocytes, partly lymphoid and plasma cells, and partly large cells derived by proliferation from the lining epithelium. Occasionally organization of the exudate is found to be taking place.

The exudation within the bronchi is composed chiefly of polynuclear leucocytes. When the inflammation is severe there is intense infiltration of the wall, chiefly by lymphoid and plasma cells. Membrane when present occurs most frequently in the bronchi of larger calibre, and is always fibrinous in character, never hyaline.

2. *Atelectasis and Emphysema.*—Atelectasis varying in extent and somewhat in character is almost always present. When most marked it is constantly associated with extensive bronchitis. It is usually much more distinctly lobular than the areas of bronchopneumonia, but large areas are frequently present involving a number of lobules.

Emphysema is constantly found adjoining the areas of atelectasis and to a less extent in connection with the areas of bronchopneumonia. It is never confined to definite areas of the lung, as are the atelectasis and the bronchopneumonia.

3. *Interstitial Tissue.*—In almost all cases the interstitial tissue of the lung is infiltrated with numerous lymphoid and plasma cells. They occur in the walls of the air-sacs, along the small veins, in the small fibrous septa, and also in the connective tissue of the pleura and around the bronchi. The process is always accompanied by bronchopneumonia or bronchitis. It is most frequently found when diphtheria bacilli are present in large numbers and are the principal infecting agents, but it is also found in equal intensity in cases in which the infection is due to streptococci and pneumococci.

4. *Necrosis and Abscess.*—Necrosis and abscess formation are not rare, and occurred fourteen times in this series of cases; as early as the third day of the disease and as late as the fifty-third. Infection occurs through the bronchi, and the foci, like the bronchopneumonia, are terminal. In some cases there is little or no inflammatory reaction around the necrotic tissue, which is often filled with diphtheria bacilli. In other cases the tissue is partly broken down and filled with degenerated pus cells and red blood corpuscles.

5. *Pleurisy.*—Where the areas of bronchopneumonia adjoin the

pleura, the pleural surface is usually slightly cloudy, and in some cases there is an evident fibrinous exudation confined to small areas. Occasionally there is an extensive fibrinous exudation covering the entire pleural surfaces.

6. *Bacteria.*—Nothing shows so well how little the character of a pathological process is influenced by the character of the micro-organism as the examination of the lungs in diphtheria. Diphtheria bacilli, streptococci, staphylococci, and pneumococci occur in connection with serous, purulent, fibrinous, and haemorrhagic exudations, necrosis, and abscess formation. The diphtheria bacilli are frequently found, and may be the cause of bronchitis with membrane formation, of purulent exudation, of bronchopneumonia, necrosis and abscess. They are often present in the lung in much greater numbers than in any other situation, and there may be but little change in the tissue around them.

Cultures were made from the lungs in 104 cases, and the diphtheria bacillus was obtained 69 times, the *Streptococcus pyogenes* 67 times, the *Staphylococcus pyogenes aureus* 37 times, and the *Pneumococcus* 10 times. In 17 of these cases the diphtheria bacillus was obtained in pure culture.

B. SYSTEMIC OR GENERAL LESIONS.

Introduction.

Systemic or general lesions are due to the absorption of the diphtheria toxin and its action in dilute form on the various cells and intercellular substances of the body. The lesions are best studied in those cases in which no general invasion with secondary micro-organisms has taken place. It is impossible of course to exclude the action of other toxins absorbed from other bacteria, such as the *Streptococcus pyogenes* which may be present in the local lesions. Fortunately, however, the general lesions found can be controlled experimentally by the inoculation of animals with the diphtheria bacillus and by the injection of the diphtheria toxin, so that we may be fairly sure of the exact pathological changes produced in man by the diphtheria bacillus.

The systemic lesions are of three kinds, (*a*) degenerative, (*b*) proliferative, and (*c*) infiltrative. The degenerative lesions affect chiefly the essential or so-called parenchymatous cells of the various organs and tissues, and do not differ in character from those found in a variety of other acute infectious diseases. The proliferative lesions are due partly to proliferation of the interstitial connective tissue cells (heart, etc.), partly to multiplication of the endothelial cells (lymph nodules everywhere), and partly to increase in number of the cells belonging to the lymphocyte and myelocyte series (lymph nodes, spleen, bone marrow). The infiltrative lesions are due for the most part to cells of the lymphocyte series invading the interstitial tissue of many of the organs (kidneys, etc.).

The Bone Marrow.

The following statement of the changes which occur in the bone marrow in diphtheria is based on the study of forty-eight non-selected cases in this series. Unfortunately it has been found impossible to compare this material with marrow obtained from normal human beings of similar ages. In all cases the marrow was obtained from the middle of the femur. In the adults the marrow was reddish with areas of yellow fat, whilst in children it was usually red, and of firm consistence, and could be removed in solid pieces. In some of the latter, however, it was grayish and softer.

Hyperplasia of the cells of the bone marrow always occurs, but it varies greatly in degree and in the cells which increase in number. It is less marked in adults than in children. The active proliferation of cells is shown by the many mitotic figures present. The changes which occur in the bone marrow may be summarised as follows:

(*a*) *Nucleated red blood corpuscles* are present in nearly all cases and occasionally are found in large numbers. They often show a tendency to occur in clumps.

(*b*) *The bone marrow giant cells* or myeloplaques vary considerably in number and occur in several forms, suggesting that active changes are taking place in them.

(*c*) *Polynuclear neutrophile leucocytes* occur in very small numbers, and are irregularly distributed among the other cells.

(*d*) *Endothelial cells* are rare. When present they betray their phagocytic tendency by the inclusion of polynuclear leucocytes and lymphocytes.

(*e*) *Eosinophile cells* are generally increased in number. Both the mono- and polynuclear forms occur, often in about equal proportions. Sometimes the eosinophile cells are present in great numbers; in one case, for example, forming half of all the cells present. In this case they were chiefly of the mononuclear variety.

(*f*) *Lymphoid cells* are always found, sometimes in considerable numbers. They are generally diffusely scattered among the other cells, but occasionally are grouped in compact masses.

(*g*) *Plasma cells* of the characteristic Unna type are sometimes present and show a tendency to form small clumps. Some of them have two, three, and even four nuclei.

(*h*) The common *myelocyte* with vesicular nucleus and slightly basophilic cytoplasm, which stains faintly with eosin in a small area near the nucleus, is usually found in abundance, but sometimes in diminished numbers.

(*i*) *Premyelocytes and Prelymphocytes.*—Besides the cells, which are sharply characterised and readily recognised, there occurs in the bone marrow in diphtheria, sometimes in great numbers, a series of undeveloped cells which unquestionably belong to two groups but which in their undifferentiated forms cannot be distinguished from each other. These two groups may be called the premyelocyte and the prelymphocyte groups or series of cells. If fully developed they would form cells which in consequence of the differentiation of the nucleus and cytoplasm could be readily recognised. In consequence of the injurious action of the diphtheria toxin and possibly also owing to the destruction of many cells in the body and the demand for the production of new ones, this differentiation of cells of the myelocyte and lymphocyte types is more or less seriously interfered with. In favour of this view is the fact that it is possible to trace all stages between these undifferentiated cells and the myelocytes on the one hand and the lymphoid and plasma cells on the other.

It seems probable that only the prelymphocyte series of cells gain access to the blood-vessels and enter the general circulation along with similar cells which are formed in the spleen, in the lymph nodes, and in the lymphoid tissue throughout the body. This view is supported by the fact that the cells which emigrate from the blood-vessels into the interstitial tissue of the kidneys and other organs often undergo complete differentiation in these organs and become easily recognised as lymphoid and plasma cells, while tpyical myelocytes are not found except in the bone marrow.

The Blood.

A well-marked leucocytosis usually occurs in diphtheria, the degree varying as a rule directly with the severity of the disease. In favourable cases the leucocytosis increases as the disease progresses, is greatest at the height of the disease, and steadily diminishes during convalescence. It may be prolonged after the disappearance of the inflammatory phenomena. In unfavourable cases it generally continues until death, though in certain very severe cases there may be a continuous decline until death. The leucocytosis has no definite relation to any other symptom, and is due largely to an increase in the polynuclear leucocytes, and also to some extent in mononuclear leucocytes of the lymphocyte series. The latter cells are found in such numbers in some of the veins of certain organs, especially the kidneys, that they appear as a very definite form of lesion.

Probably the majority of these cells come from the lymphoid tissue in the spleen, lymph nodes, and gastro-intestinal tract, and only a small proportion are formed in the bone marrow. To some extent they multiply in the circulating blood, as is shown by the fairly frequent occurrence of mitotic figures. They also proliferate after emigrating from the vessels and invading the tissues.

The changes in the red blood corpuscles are unimportant. Their number may be diminished, especially in the severe cases, after the disease has persisted several days. Marked degenerative changes do not occur: the haemoglobin diminishes directly with the number of corpuscles. These changes are less marked in cases treated with antitoxin.

The Lymphatic Tissues.

Under this heading are included the tonsils and the thymus as well as the lymph nodes distributed generally throughout the body. The lesions are for the most part of the systemic or general type due to toxins brought by the lymph and blood streams, but to some extent in the tonsils and less often in the cervical and bronchial lymph nodes are complicated by changes due to secondary invasion by various micro-organisms, especially the *Streptococcus pyogenes*. It must also be borne in mind that the tonsils and the cervical, submaxillary and bronchial lymph nodes are in the closest proximity to the primary local lesions and are exposed to the absorbed toxins in their strongest form.

1. *Lymph Nodes.*—The nodes most affected are the cervical and submaxillary, and next to them, both in the frequency and the extent of their lesions, the bronchial, intestinal, and mesenteric. The distant lymph nodes, such as the inguinal and axillary nodes, are but slightly if at all altered. Macroscopically the affected nodes are enlarged, soft, and hyperaemic; in some cases there

is extensive haemorrhage in and around the node, and in rare cases necrosis and suppuration occur. The lesions are most marked in cases of great intensity in which death takes place early. Two kinds of lesions may be recognised. In the first place there may be found (*a*) the ordinary lesions which may follow an injury of almost any description, consisting of congestion, haemorrhage, and diffuse and circumscribed necrosis. Numerous cells, not ordinarily found in the tissue, appear in combination with these processes. These new cells are derived in part by emigration of leucocytes from the blood-vessels, in part by proliferation of the lymphocytic and endothelial cells of the sinuses and lymphoid tissue. The swelling of the nodes is due chiefly to congestion, haemorrhage, and dilatation of the sinuses which contain serum, fibrin, polynuclear leucocytes, cells of the lymphocyte series, and phagocytic endothelial cells in various proportions.

There also occur (*b*) lesions which are distinctive of diphtheria, but which may be found in other infectious diseases of children, such as scarlet fever. These lesions consist in the formation, within the lymph nodules of the node, of foci which are very similar in appearance to miliary tubercles. In the formation of these foci there is a combination of proliferation, phagocytosis, and degeneration. Proliferation of the endothelial cells of the reticulum gives rise to the formation of large cells resembling the epithelioid cells of the tubercle. These cells incorporate the lymphocytes of the lymph nodules and gradually digest them. Often a dozen lymphocytes are included within a single phagocytic cell. As the lymphocytes degenerate they give rise to a varying amount of nuclear detritus, but caseation is never produced and giant cells are never formed in connection with the nodules. Sections stained with Scharlach R shows much fat within the phagocytic cells. It is difficult to determine whether the lymphocytes degenerate first and the endothelial cells proliferate to incorporate and remove them, or whether the endothelial cells multiply first and destroy normal cells. The first view would harmonise with Weigert's theory of a primary injury followed by changes of a reparative nature, but is difficult to prove in this case. As a rule these lesions form very discrete spherical nodules, but occasionally the process is scattered more or less irregularly throughout the lymph node. In the later stages of the disease the phagocytic cells undergo necrosis and the whole area appears as a mass of granular detritus. The lesion as described is sometimes complicated by haemorrhage, but more often by the formation of fibrin, which may be limited to small areas or extend throughout the focus.

2. *The Tonsils.*—The lesions in the lymphoid tissue of the tonsils differ somewhat from the lesions in the ordinary lymph nodes. The lymph nodules are large, and there is marked development of the so-called germ centres in them. Foci composed of phagocytic endothelial cells are found not only in the germ centres, but also to some extent in the surrounding lymphoid tissue. Both diffuse necrosis affecting single cells, and necrosis and ulceration extending into the tonsils from the crypts frequently occur. Extensive haemorrhage accompanied by fibrin formation and necrosis is frequently found.

3. *The Thymus.*—There is no division of the lymphoid tissue into lymph nodules and sinuses. The principal change is a degeneration of the lymphoid cells, most marked in the vicinity of the Hassel bodies, and their inclusion for the most part within phagocytic cells. Uninuclear eosinophile cells seem to be increased in number.

The Spleen.

The lesions of the spleen play but a slight part in the pathological anatomy of diphtheria. Macroscopically the spleen does not differ from the normal, except that the lymph nodules are usually more prominent. Microscopically lesions in three situations demand attention.

1. *The Lymph Nodules.*—The lesions here are identical with those found in the lymph nodules of the lymph nodes, and consist in the formation of more or less spherical masses of epithelioid cells. These cells are phagocytic and contain inclusions in various numbers. In some cases the inclusions are very numerous, but in other cases they are few. The phagocytic cells may be sharply defined or more or less fused together. In a certain number of cases fibrin is present, which appears first as fine fibrils spreading along the reticulum of the nodule, and later becomes coarse and assumes a hyaline appearance. Its presence seems to follow necrosis of the phagocytic cells.

2. *The Pulp.*—The most obvious change in the pulp is an increase in cells of the lymphocyte series, which are occasionally present in large numbers in cases of some duration. Most of these cells are typical plasma cells, but others approach more or less closely the lymphoid cells. They occur diffusely and in masses, in the latter case being usually grouped around small veins. Occasionally lymphoid and plasma cells are collected in considerable numbers beneath the endothelium of veins. Sometimes the pulp contains numerous polynuclear eosinophiles.

3. *The Arteries.*—Frequently the arteries in the lymph nodules are thickened and hyaline, and their lumina are more or less narrowed. Sections stained for fat always show numerous droplets in these areas. This form of lesion is, however, common in other acute infectious diseases.

The Gastro-intestinal Tract.

With the exception of the formation of membrane, which sometimes occurs in the oesophagus and stomach, the lesions of the gastro-intestinal tract are relatively unimportant. They consist of hyperplasia of the lymphoid apparatus with similar changes to those which occur in the spleen and lymph nodes. The slight extent of the lesions indicates that they are probably due to the action of toxins reaching the lymph nodules by way of the blood current and not to the action of toxins absorbed from the alimentary canal. There is also nothing in the character of the lesions to indicate that the toxins are eliminated by the alimentary canal.

The Heart.

The changes produced in the heart in diphtheria have received more attention than the lesions in any of the other viscera. Most of the investigations were undertaken with the view of finding pathological conditions in the heart which could explain the clinical evidences of impaired cardiac action.

Two lesions of much interest occur in the heart, the so-called acute fatty degeneration, and acute interstitial myocarditis. Mural thrombi in the heart must also be considered in this connection on account of their intimate relation to the interstitial changes.

PLATE IV.

Fig. 1. Focus of acute myocarditis due to infiltration with cells of the lymphocyte series. × 500.

Fig. 2. Acute myocarditis due to hyaline degeneration and necrosis of muscle fibres and invasion with endothelial cells and polynuclear leucocytes. × 1000.

1. *Fatty Degeneration.*—Fat occurs in the muscle fibres in over half the cases. It is best demonstrated by staining frozen sections in Scharlach R after fixation in formaldehyde. The fat may be present very diffusely throughout the heart or occur only in foci. It may appear in the form of fine granules only, or as large globules which

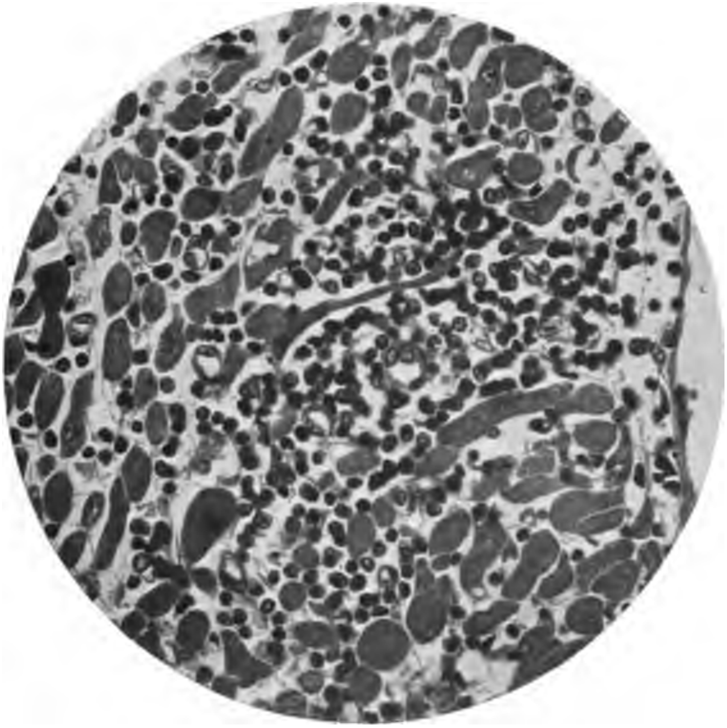


Fig. 1.

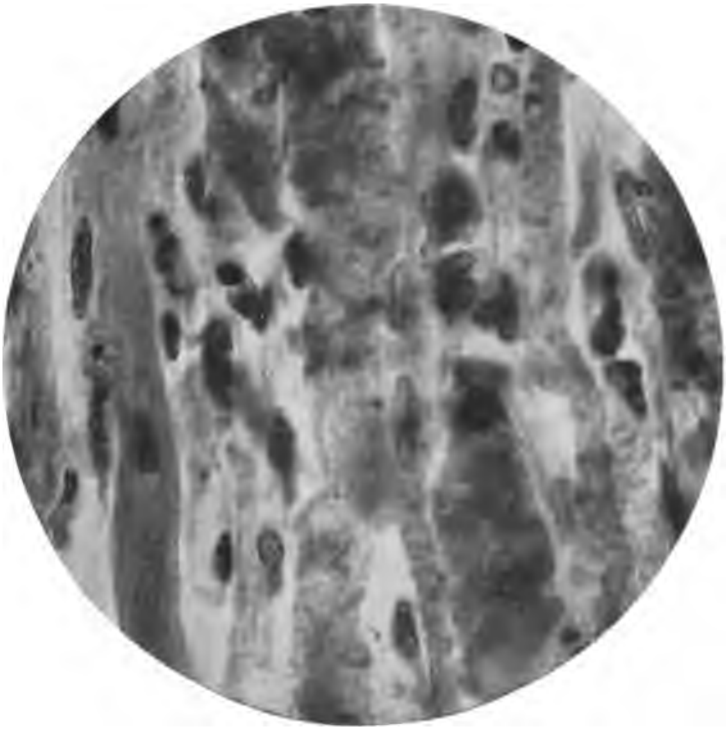


Fig. 2.

involve the greater part of the substance of a muscle cell. Fatty degeneration is in some cases the only change recognisable in the muscle fibres, but it also constantly accompanies the more advanced forms of degeneration leading to necrosis and complete destruction of the fibres. It is always more marked in the vicinity of the endocardium than elsewhere. There is but little relation between the duration of the disease and fatty degeneration, the latter being found both in cases of short and of long duration. In the cases of great severity which die early in the disease, it is generally the only lesion of the myocardium.

Segmentation and fragmentation of muscle fibres are regarded as artefacts due to poor technique, and are not included here among the other lesions. In a certain number of cases there is much more extensive degeneration leading to a hyaline change or complete destruction of the muscle fibres. This occurs in cases of considerable duration, being uncommon in those who have succumbed before the fifteenth day.

2. *Interstitial Myocarditis.*—Two forms of acute interstitial changes are found. In one there are focal collections of cells of the lymphocyte series, often typical lymphoid and plasma cells, in the tissues (Pl. IV, fig. 1). This form of lesion, which is analogous to acute interstitial nephritis, may be accompanied by degeneration of the myocardium, but is not dependent upon it. The second type of lesion consists of a hyaline degeneration and necrosis of muscle cells which are invaded by endothelial cells and polynuclear leucocytes (Pl. IV, fig. 2). In the later reparation stage proliferation of the adjoining connective tissue cells takes place.

Dr Paul A. Lewis has recently shown (results not yet published) that if the kidneys of a rabbit are removed the animal will live for two to three days. By this time numerous foci of acute interstitial myocarditis are produced in the heart, showing necrosis and almost complete destruction of the muscle fibres associated with marked proliferation of the connective tissue cells. Four to six mitotic figures are frequently seen in a single oil immersion field.

It is probable that this form of interstitial lesion in diphtheria may lead to extensive formation of connective tissue, and thereby give rise to some of the cases of fibrous myocarditis.

3. *Mural Thrombi.*—Thrombosis within the cavities of the heart is a fairly common condition in cases of rather long duration, and is due to a primary necrosis of the endocardium. The lesion is associated with the interstitial changes.

The Kidneys.

In the kidneys a variety of lesions may be found which are degenerative, exudative, and proliferative in character. The different forms of lesions may occur separately or in various combinations. The more severe forms of degeneration are found in cases of diphtheria of great intensity which die shortly after the onset. Interstitial and glomerular lesions are more common in older children and in cases of longer duration. There is no type of renal lesion peculiar to diphtheria.

1. *Degenerative changes* affect the epithelium and consist of cloudy swelling, of so-called fatty degeneration, and of transformation of the cytoplasm into hyaline droplets. In cloudy swelling the cells are swollen, and the size rather than the number of the granules in the cytoplasm is increased. Fatty degeneration is usually slight in degree. In the hyaline form of degeneration the granules normally present in the cytoplasm increase in size, coalesce, and change in staining reaction to form hyaline droplets of various sizes. A cell may contain one or many of these hyaline droplets.

2. *Acute interstitial changes* are generally included under the descriptive term acute interstitial non-suppurative nephritis, a condition which is fairly common. The lesion consists in the infiltration of the connective tissue, chiefly in the cortex, with cells of the lymphocyte series, some of which are well characterised lymphoid and plasma cells. Some of the lymphocyte cells contain mitotic figures. Occasionally polynuclear leucocytes and phagocytic cells are associated with them. The cellular infiltration is usually focal and is always most marked in three situations, at the base of the cortex adjoining the pyramids, just beneath the capsule, and around the glomeruli. In some cases the foci are confined to the pyramids. These changes in the interstitial tissue are always accompanied by the presence in the blood-vessels, especially in the upper part of the pyramids, of the same cells which emigrate out into the tissues.

In marked cases of this lesion the kidneys are greatly enlarged, while in extreme cases they may weigh four times as much as normal kidneys. The capsule is distended and thin, and, when the kidney is cut, often separates spontaneously from the surface, which is pale and of an opaque grayish colour, mottled with irregular hyperaemic areas. The stellate veins of the surface are enlarged, and punctiform haemorrhages are often found in their vicinity. On section the normal markings are obliterated, and the contrast between pyramids and cortex is less distinct. The increased size of the kidney is due chiefly to the swelling of the cortex. In the less marked cases there are opaque areas, often in lines corresponding to the course of the tubules, due to the interstitial infiltration.

The presence of these cells in the interstitial tissue does not seem to be in any degree dependent upon the degeneration of the epithelium, since the most advanced epithelial degeneration was found in cases in which no infiltrating cells were present. On the other hand marked interstitial changes may be found without degeneration of the epithelium. The degeneration which is found in foci of intense infiltration seems to depend on this infiltration, and to be due to malnutrition produced by the blocking of the blood-vessels.

In all the cases showing interstitial changes the duration of the illness was more prolonged than in those showing simple degeneration. The average duration of this series of cases was 21½ days.

3. *Acute glomerulonephritis* is much less common than the interstitial form and the lesion is chiefly of the intracapillary type due to proliferation of the endothelial cells lining the capillaries of the glomerular tuft.

Slight *haemorrhages* in the kidney occur occasionally, but haemorrhagic nephritis with escape of blood into the tubules is very rare.

The Liver.

The lesions produced in the liver in diphtheria are not characteristic and do not differ from those found in other acute infectious diseases. They are due to the effect of soluble toxic substances and not to the presence of diphtheria bacilli. The most common lesions are general degeneration of the liver cells, and necroses which are found chiefly in the centres of the lobules. The liver is generally slightly swollen, somewhat tense, and congested. Frequently the congestion is irregular, and pale, slightly yellowish foci from one to several centimetres in diameter may be seen in the dark red tissue. In some cases the liver is pale and cloudy, a condition which is due chiefly to the extent of the fatty degeneration.

1. *Degeneration of Liver Cells.*—On microscopic examination the most constant lesion is swelling of the cells with increased granulation. This process affects all parts of the lobule, but especially the centre. The liver cells are swollen, and coarse granules appear in the cytoplasm

among the uniformly fine granules normally present. A definite hyaline degeneration of the cytoplasm resulting in the formation of hyaline globules rarely occurs. Fatty degeneration is frequently present, but presents no characteristic features, and bears no relation to the other degenerative conditions of the cells.

2. *Necroses of the Liver.*—Two types of necrosis of liver cells take place in diphtheria, the central and the focal.

(*a*) *Central Necroses.*—In this form, which is the one most frequently met with, the necrosis of liver cells is confined to the lobule around the hepatic vein, extending a variable distance towards the periphery. In specimens stained with eosin and methylene blue the necrotic foci can be very easily distinguished even with a low power on account of the intense staining of the necrotic cells with eosin. The totally necrotic cells are homogeneous, hyaline, and refractive. They are small and have lost their typical shape and their connection with one another, and appear as irregular masses lying loosely in contact. A nucleus cannot be seen in these cells nor any nuclear detritus. When the necrosis is not so far advanced the cells are larger, and contain an increased number of granules, and the nucleus often forms a swollen homogeneous mass which in some cells stains intensely. In most cases the central necrosis affects all the cells in the centre of a lobule, but in others it appears only in groups of cells adjoining the central vein, while the other cells remain unaltered. The rows of necrotic liver cells are usually widely separated. This separation is not due to dilatation of the sinusoids which are usually more or less compressed and often actually occluded, but to the presence of an exudation around the liver cells, between them and the walls of the sinusoids. The exudation consists of serum, polynuclear leucocytes, and endothelial cells in variable proportions (Pl. III, fig. 2, p. 92). The necrotic liver cells are often invaded by these cells. The cause of the central necrosis is not easy to explain. Probably it is due to the action of strong toxins in the circulation, and its localisation in the centre of the lobule may be explained by the less perfect nourishment of the liver cells in this situation.

PLATE V.

Fig. 1. Peripheral nerve (popliteal) showing fat droplets in the myelin sheath. × 1000.

Fig. 2. Striated muscle (psoas magnus) showing fat droplets in three of the four fibres visible. × 1000.

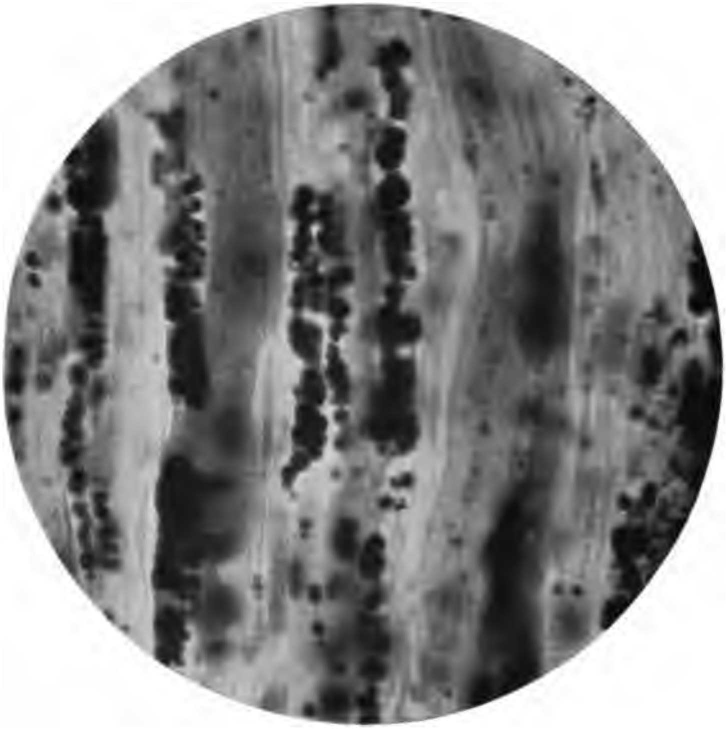


Fig. 1.

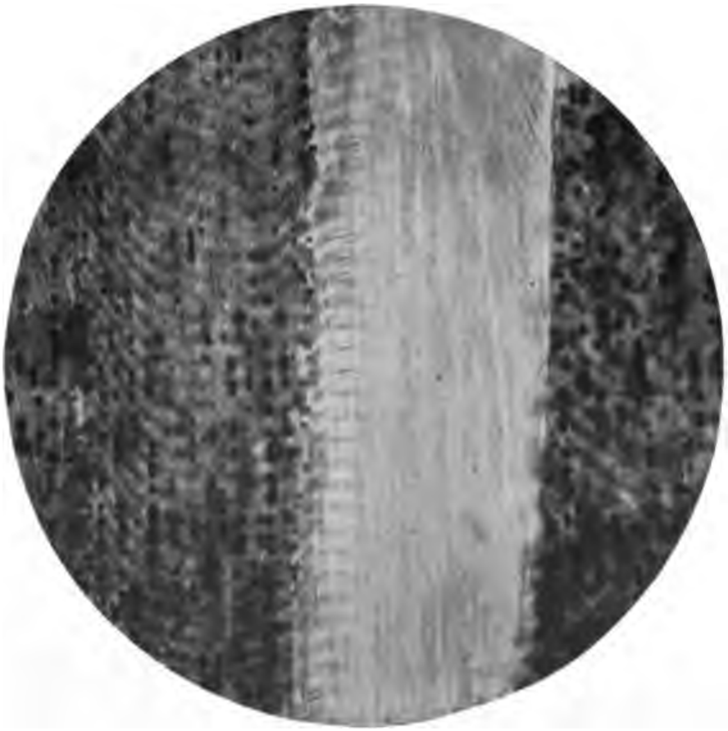


Fig. 2.

(*b*) *Focal Necroses.*—Foci of disseminated necrosis are not so common as central necroses, and the two processes are rarely found associated together. The focal necroses vary in size from areas containing but a few cells to those forming a considerable part of the lobule. The necrotic liver cells present much the same appearance as those in the areas of central necroses, and are often invaded by polynuclear leucocytes and endothelial cells. The capillaries contain very few red blood corpuscles, a variable number of leucocytes and endothelial cells, and almost always some fibrin, and the capillary walls are frequently thickened and hyaline. The necroses seem to be due to obstruction of the capillaries by cells and fibrin. In one case in which the focal necroses were very numerous, each about the size of a miliary tubercle, the liver cells in and adjoining the periphery of each area contained multiple nuclei evidently due to direct division. In this respect the lesion was analogous to the local lesions of the epithelial surfaces.

The Pancreas, Salivary Glands, Adrenals, Pituitary Body, Thyroid Glands and Testicle.

These organs show no changes macroscopically or microscopically.

The Nervous System.

In many cases of diphtheria degenerative changes take place in the nervous system, but the changes have not yet been studied in a sufficient number of cases to make a general statement possible. In this series twenty-eight cases were examined, which were selected either on account of cardiac symptoms, or paralyses, or the severity of the disease. Various nerves were stained by the Marchi method: the olfactory once; the optic chiasma twice; the third cranial nerve once; the fourth twice; the fifth twice; the sixth, seventh, eighth, and ninth, each once; the pneumogastric 28 times; the twelfth nerve twice; the phrenic twice; the splanchnic once; the ulnar once; the obturator once; the anterior crural three times; the sciatic twice. In addition the cortex cerebri was studied by the aid of Marchi's method five times; the cerebellum twice; the pons three times; the medulla four times; the spinal cord seven times; and the Gasserian ganglion once. In many cases the nerves from both sides of the body were examined, but no differences were observed.

In all 28 cases all the nerves showed various degrees of fatty degeneration, from slight to extreme (Pl. V, fig. 1). The degeneration seems almost invariably to begin in the myelin sheath. To clearly demonstrate this fact it is necessary to stain the axis cylinders in Marchi preparations by means of a rather strong mixture of acid fuchsin and picric acid. In sections stained in this way the axis cylinders are often found pushed to one side, so that the drops of fat lying in the middle of the nerve fibre and apparently within the axis cylinder are clearly shown to have originated in the myelin sheath.

As a rule the change in the myelin which causes it to stain with osmic acid in the Marchi method, begins at some point close to the axis cylinder and gradually spreads around and along it. The myelin breaks up into granules, droplets, and very irregular bizarre figures which are usually more refractive at the periphery than the centre and often show double contours. As a rule the centre of the mass stains more deeply than the periphery, but sometimes small black globules with pale centres can be seen.

The changes in the axis cylinders seem to consist chiefly of swellings which are often irregular, causing them to present a beaded appearance. As the axis cylinder swells it stains very faintly, so that it is often difficult and sometimes impossible to distinguish it. When the myelin sheaths have undergone marked fatty degeneration, the axis cylinders usually cannot be distinguished. Whether they have simply swelled up and disappeared, or have undergone fatty degeneration, is difficult to determine, but in no axis cylinder which could be positively demonstrated was there any evidence of fatty degeneration.

Five examinations of the cerebrum, two of the cerebellum, three of the pons, four of the medulla, and seven of the cord, showed everywhere slight to marked diffuse fatty degeneration of the white substance. The same change was present in the anterior and posterior nerve roots.

The results of the investigation of the nervous system may be summed up as follows: Slight to marked diffuse fatty degeneration of the nerve fibres of the central nervous system and of its peripheral extensions occurs in certain cases of diphtheria.

The Skeletal Muscles.

In one case in which the nerve fibres of the central nervous system and of the peripheral nerves showed marked fatty degeneration, the muscles of the tongue and of the ulnar side of the forearm, as well as the sartorius muscle, and the biceps of the thigh were examined by Marchi's method (Pl. V, fig. 2). All showed a marked degree of fatty degeneration, about one muscle fibre in four being affected. The fat drops were small and usually very evenly distributed throughout the whole of the affected fibres. The heart muscle in this case also showed marked fatty degeneration. In the second case in which fatty degeneration of the heart and of the nervous system was marked, the muscles of the tongue and of the diaphragm, and the tibialis anticus all showed a similar degenerative process. In places three fibres out of four were studded with fat drops. It seems probable that in all cases where fatty degeneration of the heart and of the nervous system has occurred, a similar change will be found in the skeletal muscles.

C. SECONDARY LESIONS.

In many, perhaps in all cases, the local lesions due to the diphtheria bacillus are more or less complicated by other lesions due to invasion by secondary micro-organisms such as the *Streptococcus pyogenes.* Such invasion can be demonstrated for the most part microscopically only, and is ordinarily of such minor significance that it is neglected. Occasionally, however, secondary invasion of the local lesions results in extensive destruction of the tissues, but it is impossible to state precisely how much is due to the diphtheria bacillus and how much to the pyogenic micro-organisms. The ulcerations of the tonsils, etc., already considered under the local lesions ought to be placed under this heading. In the lungs, in the accessory sinuses of the nose, and in the middle ears these secondary invaders are, however, easily demonstrated by bacteriological methods. The results of examinations of the lungs have already been mentioned (p. 102), and the results of the bacteriological examinations of the accessory sinuses and of the middle ears can be summarised briefly. When several micro-organisms are present, it is of course impossible to say which invaded the cavity first, and the same statement is true of the infections in the lungs. In all these cases it can, however, be said that it was the diphtheria bacillus which first attacked the body as a whole.

(*a*) *The accessory sinuses* of the nose were examined by means of the Hark operation in 63 cases. Acute inflammation of the antra of Highmore was found in 39, and in 24 the diphtheria bacillus was present alone or associated in various combinations with the *Streptococcus pyogenes*, the *Pneumococcus*, and the *Staphylococcus* *pyogenes aureus.* In the remaining cases the latter bacteria alone were present.

In some cases the exudate was serous or seropurulent, in others distinctly purulent, while in a few cases a fibrinous membrane was found. Seven times a mucoid secretion only was present. It seems probable that the infection of the antra of Highmore with diphtheria bacilli explains the persistence of these bacteria in cultures from the nose long after all evidence of membrane has disappeared from the throat: in some cases they have persisted for months.

In adults the sphenoidal and ethmoidal sinuses are often involved like the antra, but in children these cavities are not developed.

(*b*) *The middle ears* were examined in 144 cases and showed infection in 86, the exudate varying from mucoid to purulent. The results of cultures were as follows: the diphtheria bacillus was found 39 times, *Streptococcus pyogenes* 51, *Staphylococcus aureus* 19, *Pneumococcus* 6, in various combinations.

(*c*) *Acute abscesses* were present in 21 cases. Bacteriological examinations gave the following results.

(1) Abscesses of the cervical lymph nodes occurred in 12 cases, containing in four cases the diphtheria bacillus, in eight the streptococcus, and in three the *Staphylococcus aureus*, either in pure culture or in various combinations. (2) Retropharyngeal abscess were present in three cases. In one case a streptococcus alone was found, in another the diphtheria bacillus, a streptococcus, and *Staphylococcus aureus*, and in the third a streptococcus and *Staphylococcus aureus.* (3) One case of retrolaryngeal abscess occurred containing a streptococcus and *Staphylococcus aureus.* (4) An abscess of scalp, due to *Staphylococcus aureus*, occurred in one case, and (5) an abscess of the bronchial lymph node due to a streptococcus in one case. (6) An abscess of tonsil, containing the diphtheria bacillus and *Staphylococcus albus* was found in one case; (7) an abscess of the spleen due to a streptococcus in one case; and (8) an abscess over trochanter of femur due to a streptococcus in one case.

(*d*) *A general septicaemia* with other bacteria than the diphtheria bacillus occurs in about 30 per cent. of the fatal cases. The bacterium most frequently found is the *Streptococcus pyogenes.* It occurs both alone and associated with the diphtheria bacillus. Less commonly the *Staphylococcus pyogenes aureus* invades the blood. The order of frequency in which these bacteria are obtained by culture from the internal organs is as follows: liver, spleen, kidneys, and heart's blood.

(e) *Acute endocarditis* occurred in seven cases. From one the diphtheria bacillus was obtained, from another the streptococcus, and the other cases were not examined by culture.

(f) Five cases of *acute pericarditis* occurred, all due to the streptococcus, but in one case the pneumococcus was also present.

(g) *Acute pleurisy* was found in 23 cases, but cultures were rarely taken. The streptococcus was obtained six times, pneumococcus twice, and the *Staphylococcus aureus* once.

(h) *Acute peritonitis* occurred in two cases, both due to the streptococcus.

(i) *Acute suppurative mediastinitis* was present in two cases, and in both the streptococcus was found.

(j) *Other diseases.* Cases of diphtheria are often complicated by the action not only of various pyogenic bacteria, but also of other pathogenic micro-organisms which have invaded the body before the onset of diphtheria or during its progress.

In this series of cases the following combinations of diphtheria with other diseases were found.

Diphtheria and tuberculosis, 39.
Diphtheria and scarlet fever, 34.
Diphtheria and measles, 16.
Diphtheria, scarlet fever, and measles, nine.
Diphtheria and erysipelas, one.
Diphtheria and epidemic cerebrospinal meningitis, one.
Diphtheria and typhoid fever, two.
Diphtheria, tuberculosis, and dysentery (*Amoeba coli*), one.

D. REPAIR, CAUSES OF DEATH, SEQUELAE, AND INCUBATION.

Repair.

In the process of repair, if the necrosis in the local lesions has affected the epithelium only, the membrane gradually peels off or softens, and epithelium grows in from the adjoining unaffected edges. If the necrosis has extended into the submucosa, the necrotic tissue has to be softened, along its line of union to the living tissue, by the action of the leucocytes and replaced by scar tissue. The necrotic epithelium in the mucous glands is dissolved and removed, and then replaced by the proliferation of those epithelial cells which have escaped injury. The exudation in the submucosa is gradually absorbed.

In the systemic lesions reparative changes also take place. Fat disappears from the various cells. Hyaline and necrotic cells in the different organs are dissolved and removed. Proliferated connective tissue cells remain as dense scar tissue, as for example in the heart. The proliferated endothelial cells in the lymphoid tissue throughout the body undergo fatty changes and necrosis, and then disappear. Small hyaline masses sometimes mark the places where they have been.

The lesions due to the exudation of cells of the lymphocyte series into various organs gradually disappear either by migration of the cells into the lymphatic system or by necrosis and absorption.

The bone marrow regains its function of producing fully differentiated cells of the various types normally present in the bone marrow and in the circulation.

Causes of Death.

The common causes of death in diphtheria at the present day, when laryngeal obstruction is so readily relieved by intubation or tracheotomy, are extension of the membrane to the bronchi, bronchopneumonia, toxaemia, septicaemia, and cardiac failure. These represent the prominent or immediate causes of death, for as a rule death is due to a combination of causes.

McCollom in an analysis of 121 fatal cases of diphtheria, of which 71 came to autopsy, assigned the causes of death as follows: extension of membrane to bronchi 39; bronchopneumonia 29; toxaemia and septicaemia 29; cardiac complications 19; abscess of lung two; tuberculosis two; thrombus of the lateral sinus one.

While antitoxin treatment may lead to the disappearance of a diphtheritic membrane in the throat or bronchi, or counteract a toxaemia or septicaemia due to the diphtheria bacillus, it cannot influence a bronchopneumonia or a septicaemia due to the *Streptococcus pyogenes* or the *Pneumococcus.* Secondary lesions due to invasion by other micro-organisms than the diphtheria bacillus represent, therefore, a great source of danger.

The toxaemia, due chiefly to the absorption of the diphtheria toxin, but in some cases to the toxins of other bacteria also, is the cause of the various systemic lesions already described, fat in the cardiac and striated muscles, in the brain, cord, peripheral nerves, kidneys, liver; necrosis of heart muscle; necrosis in the liver; and degenerative and proliferative changes in the bone marrow and in lymphoid tissue throughout the body.

While a septicaemia with the diphtheria bacillus, the streptococcus, or other bacteria is probably not necessarily fatal, these organisms tend to increase the degree of toxaemia and undoubtedly play a deciding part in some cases.

The lesions of the heart in the cardiac cases, namely fat in the muscle fibres, hyaline degeneration and necrosis of the fibres, the infiltrative and proliferative changes in the interstitial tissue, and the formation of mural thrombi are all the results of the toxaemia. According to Thomas and Hibbard about one death in five is due to heart failure which occurs most frequently during the second week of the disease, but often also during convalescence, even as late as the sixth or seventh week after apparent recovery. While the early cases seem to be due to paralysis of the vaso-motor centre in the medulla, the later cases are probably the result of the changes in the heart muscle, the hyaline degeneration and necrosis which constitute the more important form of acute myocarditis.

The kidneys are rarely the immediate cause of death, although marked lesions of a degenerative, infiltrative, or even proliferative character are often present.

Sequelae.

The most common sequela of diphtheria is paralysis due to degeneration of the nerve fibres in consequence of the action of diphtheria toxin. Clinically the lesion is a toxic neuritis. The symptom occurs in 10 to 20 per cent. of all cases, more often during the second or third week of convalescence but occasionally as early as the seventh or eighth day of the disease. While the paralysis occurs more commonly during or after the severer cases, it may follow very mild cases. The neuritis usually causes paralysis of a single muscle or group of muscles, but the multiple form of diphtheritic neuritis is not uncommon. The muscles most frequently affected are those of the soft palate, next most commonly those of the vocal cords. Third in frequency come the muscles of the eyes. The muscles of the body and extremities are rarely paralysed.

A second common and very important sequela is insufficiency of the heart due to the changes which have taken place in the muscle and which may lead to heart failure. This condition has already been considered under Causes of Death.

The lesions of the kidney, although often marked, do not seem to lead to chronic nephritis as is so often the case after scarlet fever.

Incubation.

The period of incubation in diphtheria does not depend on any complicated developmental cycle of the causal agent such as is necessary with some of the pathogenic protozoa. Instead it depends chiefly on the rapidity with which the bacilli multiply and produce toxin. If a patient is infected with a thousand diphtheria bacilli instead of with only one, he will naturally show lesions and symptoms more quickly. The virulence of the infecting micro-organism would also make some difference. The time of incubation is generally stated to be two to five days, rarely longer. In the case of a student who cut his finger while removing the organs of the neck at an autopsy on a child dead of diphtheria and who then sucked his finger, symptoms and lesions were well marked at the end of twenty-four hours.

Experimental Diphtheria.

Local and systemic lesions similar in character to those occurring in man after natural infection can be produced in susceptible animals[1] by inoculation with the diphtheria bacillus, but very virulent cultures must be used in order to produce on mucous surfaces a typical local lesion with membrane formation. Ordinarily inoculations are made into the subcutaneous tissues. The resulting lesion is similar to that produced in man in the rare cases of accidental infection of cuts on the finger, etc. The diphtheria toxin apparently cannot bring about membrane formation on mucous surfaces, probably owing to the difficulty of keeping it applied to the surface for a sufficiently long time. Injected subcutaneously it gives rise to a lesion exactly like that produced by the bacillus.

The systemic or general lesions are alike except in severity in all cases. It makes no difference whether the bacillus or the toxin is used, but the use of toxin has the advantage that the amount and strength can be accurately measured and the severity of the lesions produced absolutely controlled. With the bacillus this control of the results is much less certain.

---

[1] Experimental diphtheria (see Index).

LITERATURE.

Pathology of Diphtheria in General.—’76, Bizzozero ; ’87, Oertel ; ’91, Arnheim ; 99, Baginsky ; ’01, Councilman, Mallory, and Pearce.

Diphtheritic Membrane.—’54, ’56, Virchow ; ’66, Wagner ; ’69, Cornil et Ranvier ; ’70, Nasiloff ; ’71, Hartman ; ’74, Senator ; ’78, ’80, Weigert ; ’82, Cohnheim ; ’82, Peters ; ’86, Rindfleisch ; ’87, Oertel ; ’87, Orth ; ’87, Roger et Bayeux ; ’89, Heubner ; ’91, Middeldorpf und Goldman ; ’96, Neumann ; ’97, Baumgarten ; ’98, Henka ; ’99, Baginsky ; ’01, Councilman, Mallory, and Pearce.

Diphtheria of Stomach.—’88, Smirnow ; ’95, Cronemeyer ; ’01, Councilman, Mallory, and Pearce.

General Infection with the Diphtheria Bacillus.—’89, Kolisko und Paltauf ; ’90, Babes ; ’93, Booker ; ’93, Canon ; ’93, Frosch ; ’93, Howard ; ’94, Genersisch ; ’94, Kutscher ; ’94, Wright ; ’95, Reiche ; ’95, Stokes ; ’95, Wright and Stokes ; ’96, Kanthack and Stephens ; ’96, Nowak ; ’97, Mya ; ’97, Stephens and Parfit ; ’98, Pearce ; ’01, Councilman, Mallory, and Pearce.

Diphtheria Bacilli in the Lungs.—’84, Loeffler ; ’85, Darier ; ’85, Thaon ; ’91, Johnson ; ’91, Mosny ; ’91, Strelitz ; ’92, Netter ; ’93, Booker ; ’93, Flexner ; ’93, Frosch ; ’94, Kutscher ; ’95, Belfanti ; ’95, Boarsohn ; ’95, Woodhead ; ’95, Wright and Stokes ; ’97, Horton-Smith ; ’97, Kanthack and Stephens ; ’98, Pearce ; ’01, Councilman, Mallory, and Pearce.

Blood.—’94, ’95, ’99, Morse.

Lymph Nodes.—’76, Bizzozero ; ’87, Oertel ; ’94, Bullock und Schmorl ; ’98, Bezançon et Labbe ; ’01, Councilman, Mallory, and Pearce.

Spleen.—’76, Bizzozero ; ’87, Oertel ; ’95, Bezançon ; ’95, Katzenstein ; ’96, Barbacci ; ’96, Ribbert ; ’00, Waschkewitch ; ’01, Councilman, Mallory, and Pearce.

Heart.—’70, Hayem ; ’73, Mosler ; ’77, Birch-Hirschfeld ; ’77, Rosenbach ; ’82, Leyden ; ’83, Unruh ; ’87, Oertel ; ’88 and ’90, Huguenin ; ’90, Schemm ; ’91, Romberg ; ’91, Savigne ; ’91, Welch and Flexner ; ’91, Arnheim ; ’93, Hesse ; ’94, Preisz ; ’94, Vincent ; ’94, Comba ; ’95, Schamschin ; ’95, Papkaw ; ’96, Scagliosi ; ’97, Hallwachs ; ’97, Mollard et Regaud ; ’98, Hibbard ; ’99, Romberg and Passler ; ’00, Thomas and Hibbard ; ’01, Councilman, Mallory, and Pearce ; ’04, Pratt ; ’04, White and Smith.

Kidneys.—’80, Brault ; ’83, Fürbringer ; ’83, Fischl ; ’87, Tschiglaw ; ’91, Kuck ; ’93, Baginsky ; ’93, Bernard und Felsenthal ; ’95, Katsenstein ; ’95, Reiche ; ’98, Councilman ; ’01, Councilman, Mallory, and Pearce.

Liver.—’87, Oertel ; ’93, Gaston ; ’95, Katzenstein ; ’96, Baldassari ; ’96, Barbacci ; ’01, Councilman, Mallory, and Pearce ; ’01, Mallory.

Nervous System.—’98, Thomas ; ’01, Steensland.

Skeletal Muscles.—’01, Steensland. [*Skin.*—See Index.]

Middle Ears.—’93, Councilman ; ’95, Wright ; ’96, Podack ; ’99, Baginsky ; ’01, Councilman, Mallory, and Pearce.

Accessory Sinuses of Nose.—’99, Pearce ; ’01, Councilman, Mallory, and Pearce.

Experimental Diphtheria.—’83, Klebs ; ’84, Loeffler ; ’87, Roger et Josue ; ’89, Zarniko ; ’90, Babes ; ’91 and ’92, Welch and Flexner ; ’92, Flexner ; ’93, Abbott and Ghriskey ; ’95 and ’97, Flexner ; ’97, Mollard et Regaud ; ’98, Flexner and Anderson.

SECTION IV.

By G. S. GRAHAM-SMITH.

CHAPTER IV.

THE DIPHTHERIA BACILLUS.

Introduction. Morphology of the diphtheria bacillus, in smears and in culture. Size, shape. Staining of the protoplasm. Classification. Involution form. Characters at various periods of growth on serum. Post-fission movements. Branching forms. Spores. Reaction to staining agents. Loeffler's, Gram's, Nicolle's, Neisser's, Crouch's, Coles', Bronstein's, Cobbett's, Piorkowski's, Schauffter's, Falières', Ficker's, De Rovaart's, Peck's, Pitfield's, Andrade's, Pugh's, Higley's, Ljubinsky's, Epstein's and Boni's methods. Cultivation on artificial media. Loeffler's, Lorrain Smith's, Cobbett's, Kanthack and Stephens', Hayward's, and White's serum media. Agar. Gelatin. Potato. Broth. Products of growth in broth. Action on different sugars. Effects of alkalis and acids on the growth. Milk. Litmus whey. Indol production. Vitality in culture. Chromogenic cultures. Influence of temperature on growth. Powers of resistance to cold, drying, light. Period of life in soil and dust. Effects of putrefaction. Action of germicides. Powers of resistance of bacilli enclosed in membrane. Behaviour in food substances: water, milk, butter, cheese, wine, and bread. Virulence. Occurrence of diphtheria bacilli in notified persons, and contacts, in families, amongst attendants, in hospital wards, and schools. Distribution amongst healthy non-contacts. Summary.

Introduction.

THE diphtheria bacillus belongs to a group of organisms sometimes spoken of as the Corynebacteria (Lehmann and Neumann, 1896), the members of which resemble one another more or less closely in morphology and cultural characters. Consequently the diphtheria bacillus has to be distinguished not only from species belonging to this group, but from others, which though they resemble it in morphology, differ widely from it in some of their biological characters.

All who have written on the subject of diphtheria bacilli lay emphasis on the great divergence in morphology shown by various strains which in their cultural reactions and pathogenic properties are identical. According to their morphological characters in young cultures the bacilli have been divided into morphological types. Some of these types are common, others are rare. Bacilli resembling the diphtheria bacillus more or less closely in morphology are of frequent occurrence in the throat, nose, eye, ear, skin and genital organs. These have been termed pseudo-diphtheria bacilli.

The diphtheria-like or pseudo-diphtheria bacilli in their various forms have been a source of endless confusion. Some authors have called pseudo-diphtheria bacilli all the diphtheria-like bacilli which they encountered, which did correspond in some of their cultural characters to typical diphtheria bacilli. Others have restricted the term to bacilli which in all points resemble the diphtheria bacillus except in their pathogenic action on guinea-pigs, i.e. non-virulent diphtheria bacilli. Others again, the majority among the more recent writers, restrict the term pseudo-diphtheria bacillus to the organism which will be described in the following chapters as the Hofmann's bacillus.

The subject is still further complicated by the fact that one school of investigators considers that the pseudo-diphtheria bacillus (usually meaning the Hofmann's bacillus) is merely an attenuated and slightly altered diphtheria bacillus, capable under favourable conditions of turning into the diphtheria bacillus and giving rise to diphtheria. The other school hold that this organism is in no way related to the diphtheria bacillus and is incapable under any conditions of being converted into the latter. This is a subject of more than academic interest since the Hofmann's bacillus is commonly present in the normal mouth and nose, and, if the view of the former school is well founded, must have a considerable influence on our efforts to check the disease by the isolation of persons infected with genuine diphtheria bacilli.

Another name, which has been used in various senses and has consequently added to the confusion, is the term Xerosis bacillus. By some it has been apparently used to denote the non-virulent diphtheria bacillus, occasionally found in the throat, while by the majority of writers this name is only applied to a diphtheria-like organism frequently present on the normal and diseased conjunctiva. The relationship of the latter to the diphtheria bacillus has also been a subject of dispute. The term Xerosis bacillus will be here used only to denote the diphtheria-like organism found in the eye (see Chapter X).

In order to render the subject as clear as possible the diphtheria bacillus and the organisms which resemble it will be considered under the following headings:—

(1) The diphtheria bacillus. Its morphological types, cultural and pathogenic characters and the conditions which influence its growth, vitality, and distribution (Chapter IV).

(2) The Hofmann's bacillus or pseudo-diphtheria bacillus treated in a similar manner (Chapter V).

(3) The relationship of the diphtheria bacillus to Hofmann's bacillus and the arguments for and against the identity of the two organisms (Chapter VI).

(4) Other diphtheria-like organisms which differ in some more or less important characters from diphtheria and Hofmann's bacilli will be considered in the chapter (X) dealing with "Differential Diagnosis." Amongst these is the Xerosis bacillus. The majority of these organisms have been found in other situations than the throat. Consequently it has been thought advisable to consider each in connection with the situation in which it has been found rather than in groups according to their characters. A synopsis of their principal characters is given at the end of that chapter.

Morphology of the Diphtheria Bacillus.

The diphtheria bacillus is most commonly found in the mouths and noses of those who are suffering from clinical diphtheria. It has also been found in the throats of healthy persons who have come into, contact with the patients. More rarely it occurs in lesions[1] of the eye skin, ear, and female genital organs, and on objects soiled by the saliva of diphtheria patients. Very rarely it has been found in milk and water, and a few instances are recorded of animals suffering from the disease.

1. *In smear preparations*[2] *made from false membranes* the diphtheria bacilli stain well by the ordinary aniline dyes and appear as slightly curved or nearly straight rods of various lengths usually about 3μ long,

---

[1] Examples of the rarer lesions due to the diphtheria bacillus are given at length (Chapter X).

[2] The organisms from which diphtheria bacilli have to be distinguished in such preparations are described later (Chapter X).

but they may be as short as 1μ or as long as 5μ or more. The contour is irregular, and in the majority of specimens at least one end is slightly swollen, giving the organism a wedge or club-shaped appearance. Occasionally both ends are swollen when the bacilli are dumbbell-shaped. This dilatation at the ends is not so marked in specimens seen in smears from false membranes as it is in bacilli obtained from cultures. The ends of the bacilli are usually rounded, but one end may be tapering. Occasionally examples are found with their greatest swelling in the middle and with both ends tapering. The protoplasm is generally irregularly stained and may show more or less transverse banding but some specimens stain uniformly. Darkly stained granules, situated at the ends and occasionally in the middle of the organisms, are frequently present in the protoplasm of the bacilli. When stained by methylene blue these granules show a violet tint. In such smears there is always a want of uniformity in the appearance of the bacilli as they lie side by side, but examples of most of the forms described may be met with in the same film. The bacilli usually lie irregularly scattered or in clusters, the individuals being disposed in all directions, but chains are never found. Some may be contained within the leucocytes. (Pl. XI, fig. 1.)

In *sections of membranes* the position of the diphtheria bacilli varies in different cases, but they are most frequently found in irregular clumps in the spaces between the fibrin in the superficial part of the membrane (Pl. XI, fig. 2). In this situation they may be the only organisms present. On the surface they are often found in very large numbers, but are usually accompanied by numerous other organisms of various kinds. In the deeper parts they are rarely found.

2. *In culture.* The diagnosis of diphtheria is usually made from cultures grown on one of the *serum media*, and descriptions of diphtheria bacilli usually relate to their morphology on these media. In stained preparations made from colonies 12—24 hours old growing on Loeffler's serum medium diphtheria bacilli vary greatly in appearance and may belong to several types. For the sake of convenience and clearness the appearances are described under the following headings: arrangement, size, shape, staining of protoplasm and involution forms.

Arrangement. The bacilli may occur singly, but are more usually arranged in groups of three, four, or more individuals lying side by side at a more or less open angle with each other or occasionally one individual may lie across the others. Sometimes the bacilli lie end to end, but are always at a more or less open angle with each other, resembling a circumflex accent, or the letters L or V. One small group of bacilli, the members of which are lying more or less parallel, is often found near a similar group forming a mass with interlacing ends. In large collections the bacilli are frequently thrown into irregular interlacing heaps resembling groups of cuneiform characters. The arrangement of the bacilli in the field has also been compared to Chinese letters or pine-needles lying on the ground. Chains even of moderate length are never found.

Size. Diphtheria bacilli from serum cultures vary greatly in length and have been divided according to their size into long, short, and medium length bacilli. The diameter of the bacilli varies between ·2μ and ·8μ and the length usually between 1μ and 8μ, but exceptionally even longer forms up to 13μ occur.

Shape. The individual bacilli are usually slightly curved, but greatly curved forms are frequently encountered. Straight bacilli are seldom met with.

The bacilli are seldom if ever uniformly cylindrical throughout their entire length, but are usually somewhat swollen at one end or the other, and not infrequently at both, especially in the longer forms. Slighter irregularities are generally present throughout their length. The ends are usually rounded, but specimens are occasionally met with in which one end is more or less pointed. More rarely both ends may be tapering.

Diphtheria bacilli may be classified according to their shape and size. Amongst the *shorter* forms wedge-shaped and irregular specimens are the most common. In these forms the swelling usually occupies one half of the bacillus, and is not entirely confined to the end, the other half being distinctly smaller and sometimes having a pointed extremity. Irregular forms with the swelling near the centre, or without any marked swelling are met with amongst these forms. In the *longer* forms wedge-shaped specimens are not common. The organism is gently curved and the ends are usually slightly greater in diameter than the rest of the bacillus, and one or more slight irregular swellings may be present in its length. These bacilli usually have a length of from 3—4μ. A third group consists of bacilli of about the same length or decidedly longer which have either one end or both decidedly swollen. The swollen ends are frequently twice as broad as other parts of the bacilli. Such bacilli are spoken of as *club-shaped.*

Short oval bacilli, which are young forms, may be encountered in almost any culture.

In preparations from any given culture the majority of bacilli are usually found to belong to one of these three groups, the last two of which are of more common occurrence than the first.

Staining of the Protoplasm.

Diphtheria bacilli have also been classified according to the mode in which their protoplasm stains by the common dyes, especially methylene blue. When stained by Loeffler's methylene blue solution, some portions of the protoplasm usually stain deeply and others lightly. The most typical condition is that which is usually met with in the long and club-shaped forms. In these the terminal swellings are usually deeply stained and frequently show in them violet-tinted granules. The rest of the protoplasm in this type is unevenly stained, showing alternate irregular lightly and darkly stained bands. These bands are seldom transversely placed across the length of the bacillus, but cross the long axis of the organism at various angles. Such specimens may be described as (I) *banded* or *segmented* bacilli. Another type of diphtheria bacillus is faintly stained but has irregular patches of darkly stained protoplasm in its substance. These have been termed (II) *irregular beaded bacilli.* A well-marked variety of this type is the form termed by Eyre (1899) the "sheath bacillus." He describes it as showing "a distinct sheath of faintly stained protoplasm tapering to a fine point at either extremity, and enclosing one darkly stained, elongated, lozenge-shaped aggregation of protoplasm about one-third of the length of the entire organism." According to the writer's experience some long irregularly beaded bacilli are always to be found in cultures of the sheath variety. In a third type the darkly stained parts are rounded and disposed regularly along the length of the bacillus, giving it the appearance of a short chain of streptococci. This has been described as the (III) *Streptococcal* form of the bacillus (Cobbett 1901). Of the above three types the first two are by far the most common, and are the ones described by most observers as typical of the diphtheria bacillus in culture. In a fourth type, which is less frequently met with, the bacilli are uniformly and darkly stained throughout their length. If not too darkly stained a narrow lightly staining septum may, however, often be seen in bacilli of this type. These are usually described as (IV) *uniformly* or *solidly* stained bacilli. Lastly (V) *oval* bacilli uniformly stained or with a light central band (very young forms) may sometimes be seen amongst the other forms.

Although oval forms are to be met with in pure cultures of diphtheria bacilli they usually represent only young bacilli (see p. 133), and virulent cultures composed of these forms alone have very rarely been encountered. That they may occasionally, however, be the only forms present in virulent cultures is proved by the experience of Wesbrook, Wilson and McDaniel (1900) during an outbreak at Owatonna.

The darkly stained violet granules (Polar bodies) mentioned above may be met with in all of these types except the very young forms.

Classification of Diphtheria Bacilli.

One of the most satisfactory methods of classifying diphtheria bacilli is that which has just been given, according to their staining properties irrespective of the size of the organisms. By this method five types may be recognised. (Cobbett, IV. 1901, p. 240.)

I. Segmented bacilli. (Pl. VI, figs. 3, 4, 5.)

II. Long, faintly stained, irregularly beaded bacilli. (Pl. VI, fig. 1.) The "Sheath" bacilli belong to this type.

III. Regularly beaded bacilli. Streptococcal forms. (Pl. VI, fig. 2.)

IV. Uniformly stained bacilli, sometimes with narrow median light bands. (Pl. VI, fig. 6.)

V. Oval bacilli, which frequently possess one unstained septum. Young forms.

Many authors have only distinguished between long, medium length, and short diphtheria bacilli, whilst others, using the length of the organisms for their main divisions, have subdivided them according to their staining reactions.

Further Classification of Diphtheria Bacilli.

Wesbrook, Wilson and McDaniel (1900) have introduced a classification which has been very frequently made use of by other observers in describing the bacilli which they have encountered. These authors say "attempts to classify types of bacilli on the appearance of microscopic fields of presumably pure cultures had to be abandoned, and, instead, we were compelled to adopt a classification based on the morphology of individual bacilli. In the study of the morphology of pure cultures in most instances, especially where they have been derived from typical clinical cases of diphtheria, it is the exception to get even a moderate degree of uniformity in the general shape, size, staining

PLATE VI

DIPHTHERIA BACILLI FROM SERUM CULTURES.

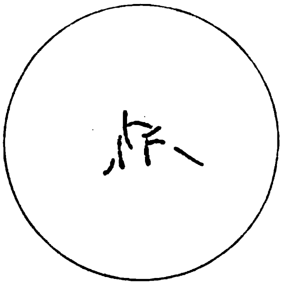


Fig. 1.
Irregularly beaded form.
Subculture 24 hours.

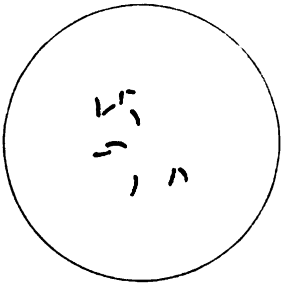


Fig. 2.
"Streptococcal" form.
First culture 24 hours.

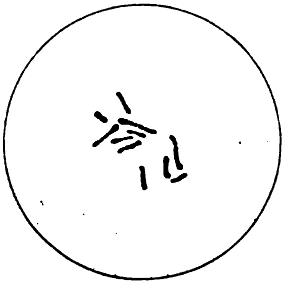


Fig. 3.
Segmented form.
First culture 24 hours.

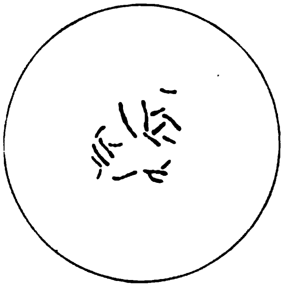


Fig. 4.
Segmented form.
First culture 24 hours.

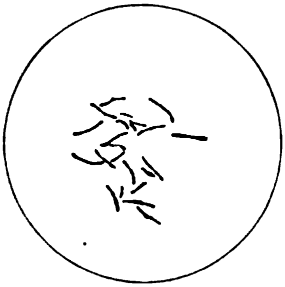


Fig. 5.
Thin segmented form.
First culture 24 hours.

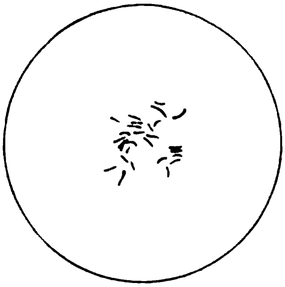


Fig. 6.
Uniformly stained form.
Subculture 24 hours.

All figures drawn with the aid of a camera lucida (Zeiss $\frac{1}{12}$ im., No. 4 oc.)
stained with Loeffler's methylene blue (diluted 1 : 5).

[From Graham-Smith, *Journ. of Hygiene*, Vol. IV. Plate XIV. 1904.]

reactions, etc., of the individual bacilli, whilst to get complete uniformity is not to be hoped for. At first it seemed possible by obtaining an accurate description and coloured sketch of all the bacilli in each pure culture to arrive at a classification. Upon becoming gradually accustomed to close observation, and particularly when it came to sketching the forms, it was soon found that one or more forms might be common to several cultures whose general morphological characteristics seemed to be quite different. By general morphological characteristics is meant the general picture seen by a trained observer. In ordinary routine examinations the observer is influenced perhaps more than he always realises by the presence of certain forms which meet his particular views in regard to type, and may overlook or minimize the importance of other to him less well-known forms which may be present."

The arrangement they adopt is a purely arbitrary and provisional classification of the morphological types (stained with Loeffler's methylene blue) and does not take into account the biological and pathogenic properties of the organisms. Their types are divided into three main groups depending on their staining reactions. (Plates VII, VIII.)

Group I. Granular Forms. These show distinct spherical, ovoid, or markedly rounded granules. These forms are represented by the plain letters, *A* to *G*. In each variety except *B* the granules show metachromatism—i.e. take on a reddish tint with fresh Loeffler's methylene blue. The granules are, as a rule, at one or both poles of the bacilli, though they may occur elsewhere also. The protoplasm of the bacilli not included in the granules varies in intensity of staining, though it is always lighter in colour than, and usually in marked contrast to, the granules.

Group II. Barred Forms. Irregularity of staining is the distinguishing character. Bacilli belonging to this general class present a distinct barred appearance as of actual removal of segments of protoplasm. The darkly staining portions vary in the intensity of the colouration in different bacilli of the same variety and even in the same bacillus. Their size and shape are variable, though, as a rule, not spherical, nor ovoidal, and the colour, whilst usually dark, does not suggest metachromatism. The number of dark segments varies from three to nine. A^1, B^1, C^1, are familiar types of diphtheria bacilli.

Group III. Solid Colour Forms. These include not only the solidly or uniformly staining forms of all shapes and sizes, but also those with the appearance of diplo-bacilli (A^2, B^2, C^2, D^2, E^2, G^1).

Types in Detail.

Types A, A^1, A^2 have often been described as involution forms, but are frequently to be found in smears and fresh cultures. (3—6μ.)

Certain of them, as type A^2, if found as the only microbe present, besides staphylococcus or streptococcus, in a throat culture would not suggest a diagnosis of diphtheria. (Pl. VII, figs. 1, 2, 3.)

Types B, B^1, and B^2. These types are long and slender. (3—7μ.) "*Type* B has been found as almost the sole diphtheria-like organism present in a series of cultures from a case of typical clinical diphtheria and proved to be highly virulent to guinea-pigs." (Pl. VII, figs. 4, 5, 6.)

"*Types* B^1 and B^2 have not been found as the predominating forms in direct throat and nose cultures, though sometimes present in small numbers. It will readily be seen from the resemblance in size and shape of *type* B^2 to various common bacteria of the mouth that it would be very unsafe to diagnose this type as *Bacillus diphtheriae* except when it occurs as a variant in pure cultures."

Type C. Often somewhat curved forms, in which the protoplasm stains light blue except always at the poles and occasionally at one point between the poles, where rounded, rarely ellipsoid metachromatic granules occur (3—6μ). This form is the most common one met with in clinical cases. (Pl. VII, fig. 7.)

C^1 has been frequently met with in clinical diphtheria (3—6μ). (Pl. VII, fig. 8.)

C^2 "Infrequently met with, but up to the present time never found by us as the predominating form even in pure cultures which had long been under study. Its virulence to man and animals is therefore a matter of conjecture" (3—4μ). (Pl. VII, fig. 9.)

Type D. Similar in most respects to C, but shorter and thicker and apparently straighter. (2—3μ.) (Pl. VIII, fig. 1.)

D^1. Shorter, straighter, and with fewer bands than C^1 (2—3μ). (Pl. VIII, fig. 2.)

D^2. Stain an intense even blue. Metachromatism is absent (1—2·5μ). This form probably represents the organism usually spoken of as Hofmann's bacillus. At Owatonna, however, a diphtheria bacillus of this shape was often present alone in clinical cases and was pathogenic for guinea-pigs. (Pl. VIII, fig. 3.)

Type E. Shorter than D, otherwise similar (1—5μ). (Pl. VIII, fig. 4.)
E^1. „ „ D^1 „ „ (1·5—2μ). (Pl. VIII, fig. 5.)

Type E^2. Very like D^2 except that it is shorter and apparently

PLATE VII

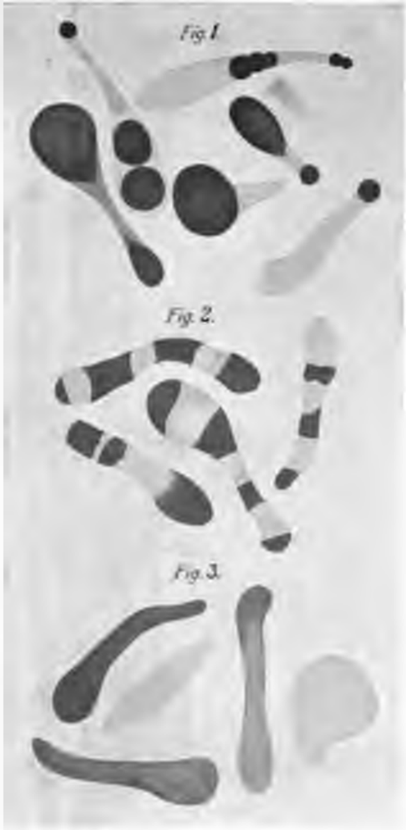


Fig. 1. Type A ⎫ 1 to 2 μ thick.
,, 2. ,, A_1 ⎬ 3 to 6 μ long.
,, 3. ,, A_2 ⎭

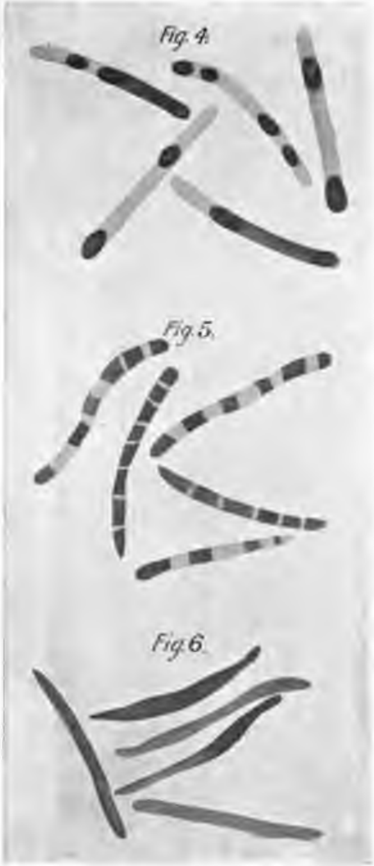


Fig. 4. Type B ⎫ 0·5 μ thick.
,, 5. ,, B_1 ⎬ 3–7 μ long.
,, 6. ,, B_2 ⎭

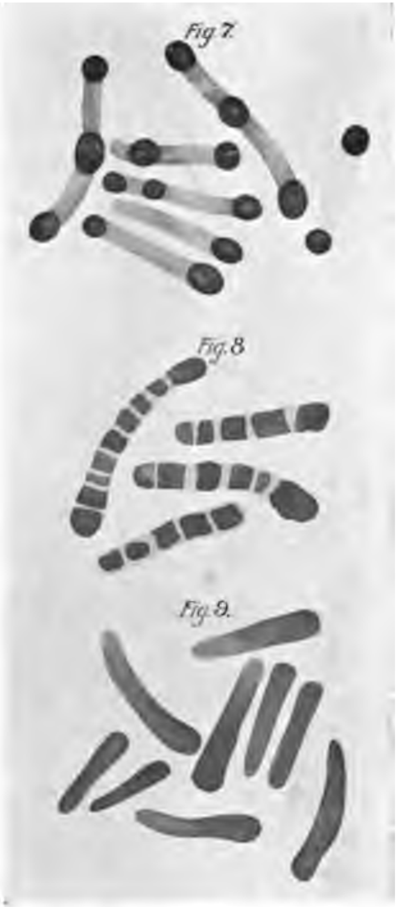


Fig. 7. Type C , 0·5–1 μ thick, 3–6 μ long.
,, 8. ,, C_1, ,, ,, ,, ,,
,, 9. ,, C_2, ,, ,, 3–4 μ ,,

Photographs from Wesbrook, Wilson and McDaniel's (1900) coloured Plates 1, 2 and 3.

PLATE VIII

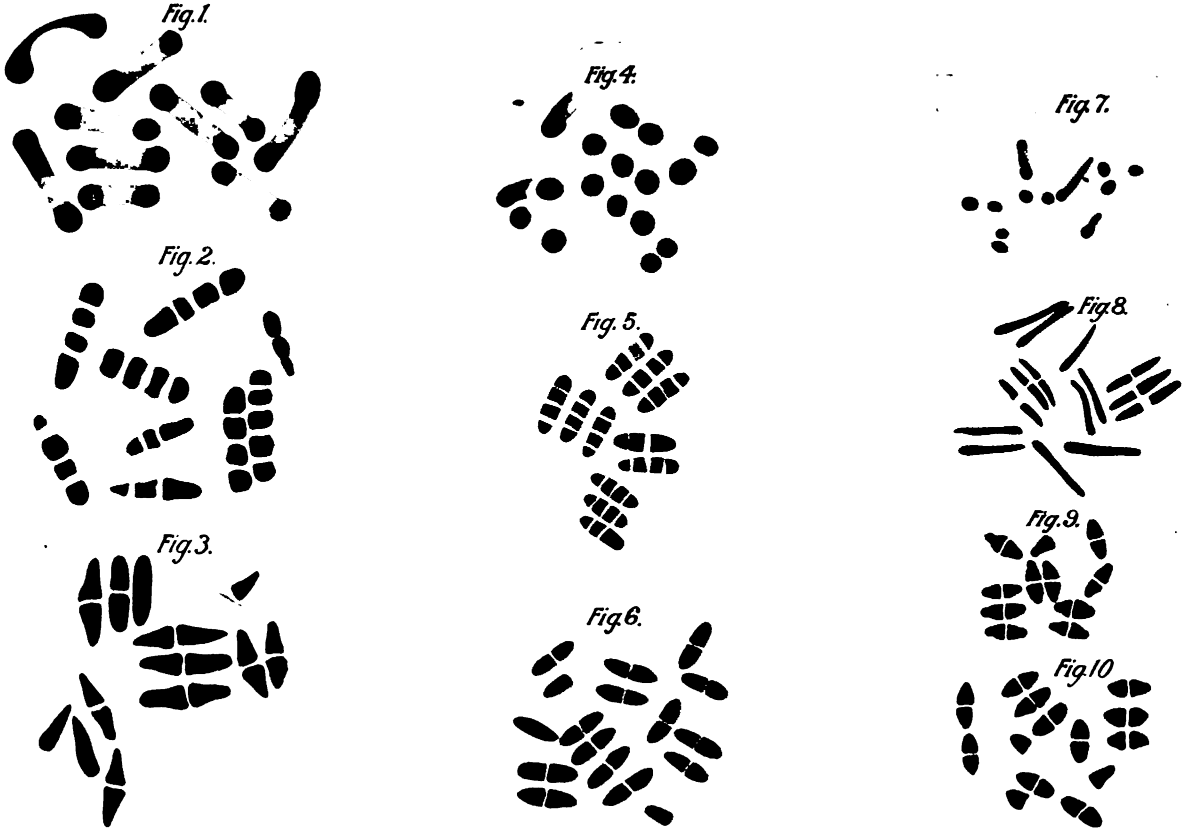


Fig. 1. Type D, 0·75–1 μ thick, 2–3 μ long.
,, 2. ,, D_1, 0·5–1 μ ,, ,, ,,
,, 3. ,, D_2, 0·75–1 μ ,, 1–2·5 μ ,,

Fig. 4. Type E, 0·5–0·75 μ thick, 1·5 μ long.
,, 5. ,, E_1, ,, ,, 1·5 μ–2 μ ,,
,, 6. ,, E_2, ,, ,, 1–2 μ ,,

Fig. 7. Type F, 0·25–0·5 μ thick, 1–2 μ long.
,, 8. ,, F_1, 0·25 μ ,, ,, ,,
,, 9. ,, G, 0·5–0·75 μ ,, 1–1·5 μ ,,
,, 10. ,, G_1, ,, ,, 1–1·25 μ ,,

Photographs from Wesbrook, Wilson and McDaniel's (1900) coloured Plates 4, 5 and 6.

straighter. Probably usually spoken of together with D^2 as Hofmann's bacilli. Not known to be pathogenic (1—2μ). (Pl. VIII, fig. 6.)

Type F. Small (1—2μ) slender bacilli with tapering extremities, stain light blue, and contain one or two metachromatic granules, at one end or near the middle or at both places. "This form is extremely rare, and its pathogenesis has not yet been studied." (Pl. VIII, fig. 7.)

F^2. Like F in shape, stains an even light blue, frequently with a central light band. "A rare form, and one which like B^2 would not be recognised as related to diphtheria in throat cultures, but only so when found as a variant in pure cultures" (1—2μ). (Pl. VIII, fig. 8.)

Type G. Shortest (1—1·5μ) granular form, usually with central light band. "The granules are very small, difficult to see and occur only at the distal extremities of the pair. It has been rarely found in clinical cases, though its pathogenesis for guinea-pigs has been shown in cultures from a non-clinical case." (Pl. VIII, fig. 9.)

G^2. A bacillus resembling D^2 and E^2 in staining and arrangement but shorter (1—1·25μ). (Pl. VIII, fig. 10.)

Reference to the observations of Denny on the differences in morphology at various periods of growth and to the observations of Hill and others on the mode of division shows that absolute uniformity of morphology in culture cannot be expected, since the various bacilli in the culture are not of the same age. Nevertheless most cultures have an individuality of their own, which may be preserved for many generations. Moreover it is frequently possible to recognise cultures with marked peculiarities derived at various times from the same individual or from persons infected by him. For descriptive purposes the general impression given by the culture to a trained observer is therefore usually sufficient without the elaborate and confusing tables necessitated by Wesbrook's method of describing a culture by the enumeration of all the various types met with in it.

Another important mode of classifying diphtheria bacilli is according to their virulence for animals (see p. 179).

Involution Forms.

After prolonged growth on a suitable medium, or more quickly on an unsuitable medium, diphtheria bacilli become considerably altered in shape[1]. Their appearance becomes more irregular, and very large forms

---

[1] The morphological appearances of the bacilli when grown on various media are given together with the microscopical appearances of the growths on these media.

PLATE IX.

Photographs of stained preparations of diphtheria cultures (from Denny, 1903). The magnification in all cases is the same (× 2000), and all the preparations were made from growths on serum and stained with Loeffler's methylene blue (Grübler) 1–3 minutes without heat.

Fig. 1. *B. diphtheriæ*, 5 hours at 36° C. This shows only uniformly staining forms. (Denny, Plate VII, fig. 1.)

Fig. 2. *B. diphtheriæ*, same culture, 8 hours at 36° C. This shows also uniformly staining forms, many of them with parallel arrangement. (Denny, Plate VII, fig. 2.)

Fig. 3. *B. diphtheriæ*, same culture, 15 hours at 36° C. The bacilli stain unevenly and show large granules. (Denny, Plate VII, fig. 4.)

Fig. 4. *B. diphtheriæ*, same culture, 48 hours at 36° C. Shows long forms with large dark staining areas. (Denny, Plate VII, fig. 6.)

Fig. 5. *B. pseudo-diphtheriæ* (Hofmann), 11 hours at 36° C. This shows uniformly staining forms like young *B. diphtheriæ*, but shorter, thicker, and with more rounded ends. (Denny, Plate IX, fig. 11.)

Fig. 6. *B. pseudo-diphtheriæ*, same culture after 24 hours. Comparison with fig. 5 shows diminution in size in the older culture. (Denny, Plate IX, fig. 12.)

Fig. 7. *B. xerosis*, 12 hours at 36° C. This shows only uniformly staining forms. (Denny, Plate IX, fig. 13.)

Fig. 8. *B. xerosis*, same culture after 24 hours at 36° C. This shows the segmented or barred forms which are characteristic of pure cultures of this species. (Denny, Plate IX, fig. 14.)

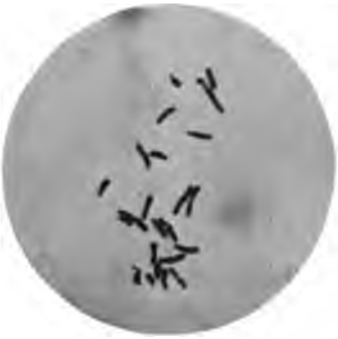


Fig. 1.

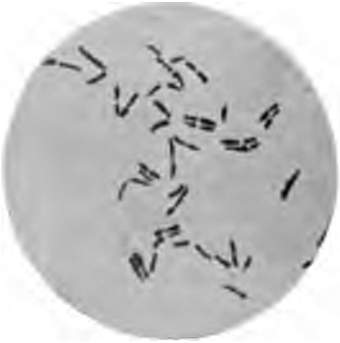


Fig. 2.

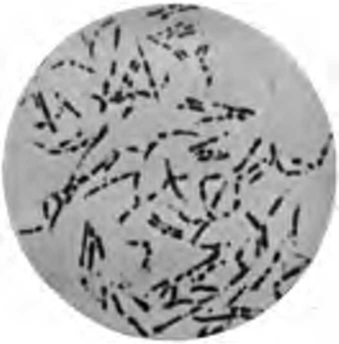


Fig. 3.

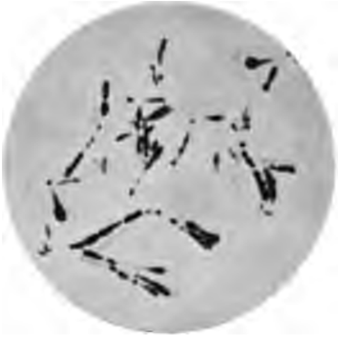


Fig. 4.

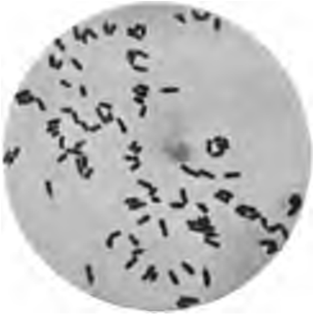


Fig. 5.

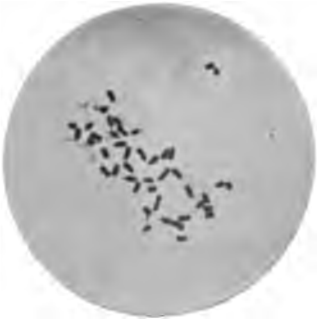


Fig. 6.

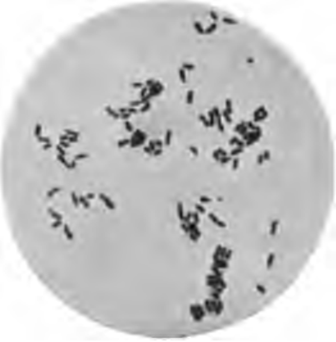


Fig. 7.

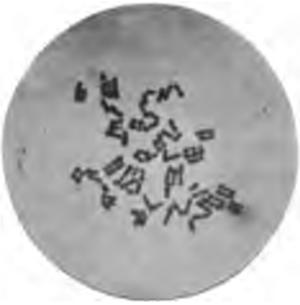


Fig. 8.

are frequently encountered. Many become greatly swollen at their ends and develop enormous club-shaped masses which stain deeply, while the rest of the bacillus may stain badly or have irregular patches of deeply stained protoplasm in it. Others become pear-shaped, or globular, while some retain their general shape but become thicker throughout. Others again show large globules at their ends while the rest of the rod appears as a faintly stained line. Specimens which take up the stain very badly are common. Some bacilli may be represented by small round masses like cocci or by a chain of such masses when they look like streptococci. In fact under such conditions every variety of shape and form and staining capacity may be met with. (Pl. VII, fig. 1, Pl. IX, fig. 4, and Pl. XI, fig. 4.)

Morphological Characters of the Diphtheria Bacillus at various periods of its growth on Serum.

Denny (1903) has recently investigated the morphological characters of 10 strains of diphtheria bacilli at various periods of their growth on serum by means of stained preparations. He inoculated serum tubes and incubated them at 36° C., and made smear preparations from them after 4, 8, 12, 15, 24, 36 and 48 hours' growth.

After 4 *hours'* growth he found the bacilli to be uniformly stained, resembling Hofmann's bacilli in shape, and frequently lying in pairs. There was considerable variation in size and shape, and some of the rods were distinctly curved. At this period they correspond to C_2 and D_2 of Wesbrook's types (Pl. IX, fig. 1).

After 8 *hours'* growth the rods are more uniform in size, still uniformly stained, and arranged in pairs. Small granules or polar bodies are occasionally to be seen in them (Pl. IX, fig. 2).

After 12 *hours'* growth the rods are longer, frequently contain granules, and often have faintly stained portions at their ends. They correspond to the *C* and *D* types of Wesbrook.

After 15 *hours'* growth the bacilli are longer and larger, and segmentation of the protoplasm is moderately well marked. The polar bodies are larger and more abundant (Pl. IX, fig. 3).

After 24 *hours'* growth uniformly stained specimens are few. The bacilli are longer, clubbing is a marked feature, and the majority show polar bodies. Segmentation is well marked.

After 36 *hours* the above appearances are still more marked. The bacilli are longer and many stain poorly.

After 48 *hours* very long forms are numerous and a considerable proportion stain badly (Pl. IX, fig. 4).

Post-fission Movements, studied in Living Cultures.

Hill (1901, 1902) in a series of investigations made observations on the development of living diphtheria bacilli and compared their mode of division with that of various other species. The observations were made by a method which he devised and called the "hanging block" method (1902). Living bacilli are spread on the surface of a sterilized cube of agar, and the inoculated surface is applied to the under surface of a cover-glass. This method allows the organisms to grow horizontally without restriction, and they are all necessarily in optical contact with the cover-glass. Working by this method he has described the post-fission movements of diphtheria bacilli (1901). "Restricting the term fission to the separation of the protoplasm of the bacterium into two portions, each capable of further growth, development, and reproduction, a striking difference as regards fission between *B. diphtheriæ* and its allies on the one hand, and *B. typhosus* (taken for the purpose as a type of a large group distinct from the diphtheroid group) on the other, lies in the post-fission movements. It may at times be possible to detect in *B. diphtheriæ* segmentation of the protoplasm previous to post-fission movements, a hard dark line, not necessarily central, developing across the axis of the rod; sometimes more than one such segmentation line is visible in a single rod. What may usually be observed is a gradual enlargement of the bacterial cell in both diameters accompanied often by slight changes in outline suggesting some plasticity of the bacterial walls; then a sudden snapping across of the rod resulting in the two portions lying at an angle with each other and suggesting for such organisms the convenient term 'snapping group.' Subsequent growth results in the gradual approximation of the distal ends of these two rods to each other, giving a parallel arrangement. The proximal ends of the new rods (those which before snapping were continuous with each other) remain contiguous to each other after parallelism is achieved. In such movements, one or both rods necessarily describe parts of a circle, and when, as often happens, the two rods are not of the same length, the shorter usually moves through a much greater arc than the longer. Subsequent growth does not usually result in the separation of the two portions at the proximal ends, the increase in length in the longer portion apparently carrying the other portion with it. That something unites the proximal ends seems further evident, because if two rods thus derived be disturbed, as by a current of water, so that they drift off, turning over and over, their relative positions remain unchanged. A single rod may break up into three portions instead of two."

The hypothesis offered by Hill to explain these movements is, in brief, as follows:—"that the visible bacterial rod is surrounded by an invisible or scarcely visible membrane (even in crowded unstained preparations, individuals lying side by side are rarely if ever in visible contact, an interval remaining always between them); that in *B. diphtheriæ* only incomplete rupture of this membrane after fission occurs, the line of rupture running round only a portion of the circumference of the original rod, leaving a bridge connecting the new rods formed; that the enlargement of these rods before snapping and the tension thus produced originates the rupture, and that the preliminary angular position, and the parallelism finally achieved are due to the pressure of the proximal ends of the new rods on each other in composition with the restraint exercised by the unruptured strap-hinge like portion of the original membrane" (Plate X, fig. 1).

The fission and post-fission movements of *B. typhosus* for example are quite different. Hill describes them as follows:—"In this species fission is always clearly evidenced by a sharp separation of the protoplasm of the original rod into two usually equal portions by a translucent segmentation interval. Later a slight curve to one side is observed in one of the two new rods still in line with each other. This curve tends to straighten afterwards, the straightening being achieved by the proximal end of the curved rod slipping slowly out of line with the other rod, towards that side on which was the convexity of the curved rod. The two rods, now free at both ends continue to grow, the proximal ends passing each other in opposite directions, and finally reaching, if nothing prevents, to the distal ends." Species such as *B. typhosus* he terms the "slipping" species (Pl. X, fig. 2).

Plate X, figs. 3, 4 show the development of single specimens of *B. diphtheriæ* into four and eight daughter cells as has just been described. The relations of the various daughter cells to one another and the time which elapsed between the various stages are given.

These movements have also been described by Kurth (1898) and Nakanishi (1901).

Hill and Rickards (1903) investigated the post-fission movements in 18 species of bacteria, namely *B. anthracis, megatherium, coli, typhi, pestis, ramosus, indicus, fluorescens liquefaciens, mucosus capsulatus, pyocyaneus,* the bacillus of logwood, Whipple's *C* and *D* and *Sp. choleræ*

PLATE X.

The figures in this plate are from Hill 1901 (*30th Annual Report of the Health Department of the City of Boston*, p. 81) and are described in his own words.

Drawings made from direct observation of bacilli, developing in hanging block preparations, under the microscope, at 37° C., warm stage. The individuals in each figure (1–4) are drawn to a common scale, but the scale was not the same for all the different figures. The diphtheria bacilli described were derived from cultures obtained in the course of routine diagnostic work of the Boston Board of Health, and were typical in morphology, cultural reactions and virulence. The typhoid bacilli observed were derived from the culture used in the laboratory for the Widal test.

Fig. 1. Actual. *B. diphtheriæ*, illustrating "snapping" (A) became (B) suddenly, the change occurring under the writer's eye; (B) became (C) gradually, as is the rule in this species. Fig. 1. Hypothetical. *B. diphtheriæ*, illustrating the writer's hypothesis of membrane rupture, devised to account for the snapping and subsequently achieved parallelism.

Fig. 2. Semi-diagrammatic. *B. typhosus*, illustrating the definite fission (B), gradual slipping aside of proximal ends (C–D) and subsequent parallelism (E, F), characteristic of this and some other species. This method is illustrated here to serve as a contrast to the snapping of *B. diphtheriæ*.

Fig. 3. *B. diphtheriæ*, illustrating development of a rod (A) into four daughter cells (G); (D) became (G) in 45 minutes.

Fig. 4 illustrates branching by apparent budding. The form (A) gave rise to nine daughter cells (H), of which eight were derived by the ordinary processes of fission, accompanied by ordinary post-fission movements. The ninth and largest cell arose as shown (A–D), by gradual enlargement of the original branch. At (E) the branch "snapped" suddenly to one side on its stem (12); became faint, losing its density and plumpness (F–H). Time relations: A to B = 37 min.; B–C = 32 min.; C–D = 16 min.; D–E = 8 min.; E–F = 62 min.; F–G = 37 min.; G–H = 38 min.; total A–H = 3 hours and 50 minutes.

Fig. 5. A, J–O show branching in the absence of metachromatic granules. G–H show metachromatic granules in the absence of branching and B–F metachromatic granules in combination with branching. In fig. C a granule is situated at the base of a branch. This condition is more frequent than might be supposed from these plates.

The genealogical relationship of the new cells in figures 3 and 4 are indicated by a modification of Ricard's system for culture record (B. R. Ricard, A system of recording cultures of bacteria genealogically for laboratory purposes, *30th Annual Report of the Health Department of the City of Boston*, 1907, p. 75).

PLATE X

Fig. 1.

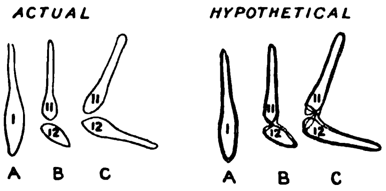


Fig. 2.

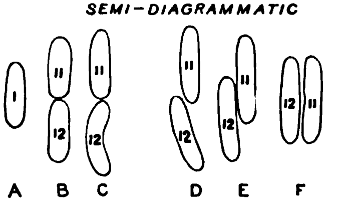


Fig. 3

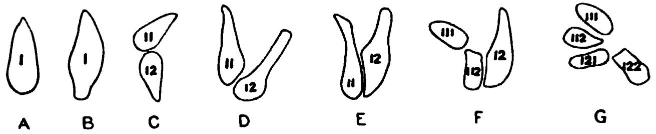
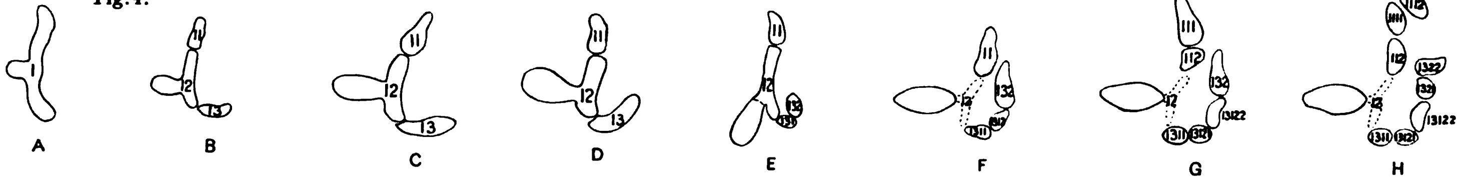
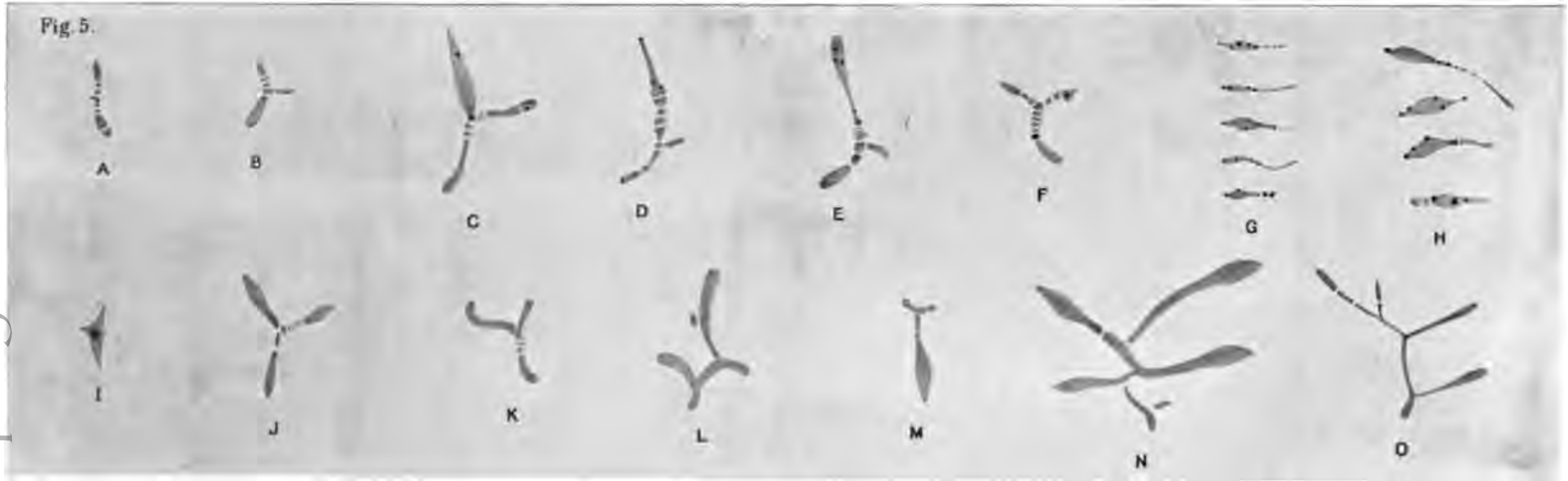


Fig. 4.





asiaticæ, as well as *B. xerosis* and *B. pseudodiphtheriæ*. They found snapping to occur only in the last two species and *B. diphtheriæ* and believe that snapping movements distinguish this group on the one hand from "typical bacilli" on the other.

Branching Diphtheria Bacilli.

Branching specimens of diphtheria bacilli have been met with in cultures and recorded by many observers, and Kanthack (12. IX. 1896) gives a short review of the observations made on this point up to 1896.

The principal hypotheses put forward in regard to branching are as follows:—

(1) That the branching is only apparent, and due to the accidental apposition of bacilli. The observations of Hill (1899, 1900 and 1902), Abbott and Gildersleeve (1903) and others have proved, however, that true branching does occur.

(2) That the bacillus pushes out a portion of its protoplasm which may constrict off or branch further without division (Pl. X, fig. 4). The researches of Hill (1902) prove that this form of branching occurs.

(3) That the bacilli are really made up of several individuals and that branching occurs when one grows out of the axial line. One of Hill's (1902, Pl. II, fig. 8) figures lends some support to this view.

(4) That branching is connected in some undefined way with the polar bodies[1]. (See Pl. X, fig. 5 C.) Babes (1895, Pl. X, fig. 3 A) pointed out that branches sometimes originate at points in the bacilli marked by polar bodies. Abbott and Gildersleeve (1903, p. 275), noticed that buds were often given off from points in the cells that were marked by the presence of irregular darkly stained masses, not the polar bodies.

(5) That branching is merely an exaggeration of the normal nodosities. Hill (1901) has shown that in many cases of branching this is the case.

(6) That branching is a degenerative change consequent upon growth under unfavourable environment.

As a result of his studies on hanging block and stained preparations Hill (1902) thinks that the following conclusions are justified in regard to branching:—

(1) Branching is not an accidental optical illusion.

---

[1] Branched forms with polar bodies are shown in Pl. X, fig. 5, A–F, and similar forms without polar bodies in fig. 5, I–O.

(2) Passive degenerative changes may give rise to slight irregular changes which simulate branches (Hill, 1902, Pl. II, fig. 7).

(3) As a part of the active development of the diphtheria bacillus, active branching by apparent budding, ending in the protrusion of an oval or elliptical body probably itself capable of further development and the production of new rods, may occur in young cultures, the parent stem then degenerating (Pl. X, fig. 4).

(4) As a part of the active development of the diphtheria bacillus, branching similar to that described, but terminating in an ordinary diphtheria rod-like body, without any degeneration of the parent stem at the point of origin, may occur on serum or agar within 17 hours, and this new rod may segment in the ordinary way or itself produce branches terminating in rods similar to itself, or in oval bodies such as above described (Pl. X, fig. 5, A—F, I—O).

(5) Various modifications of the processes described may exist.

The conditions under which branching occurs have been carefully observed by Abbott and Gildersleeve (1903). They find that it is not constant on any of the standard media, on which only a few of the organisms at any time show it. It is seldom observed on agar, potato, or broth. The presence or absence of oxygen does not affect it. They were unable to find any method which regularly ensures evidence of branching except in a very small proportion of the cells in a culture. Branching was most frequently obtained by these observers by cultivation at 37—38° C., on a solidified mixture of three parts of thoroughly beaten whole egg and one part of peptone broth containing 2% of glucose. On this medium, when slightly alkaline, 10 strains of virulent diphtheria bacilli grew well, and in nine of these branching was fairly frequently seen. When the medium was made acid, branching was more frequently seen together with degeneration of the cells in the culture.

From the alkaline medium one branching form was met with in every three or four cover-glasses, whereas from the acid medium about every cover-glass contained one.

Hill (1900) investigated the frequency of branching forms in 648 positive cultures from cases of diphtheria and found them in 64 (10%). He (1899) is convinced from his researches that at least 50% of diphtheria cultures contain a few branching diphtheria bacilli.

Bernheim and Folger (1896) remark that they have occasionally encountered membranes rich in branching forms, but from these membranes they were unable to obtain colonies on serum.

As a result of their experiments Abbott and Gildersleeve agree with Migula (1900) and regard branching as evidence of degeneration and think the phenomenon of branching does not warrant diphtheria bacilli being classified with the hyphomycetes in which group they have been placed on this account by Lehmann and Neumann (1896).

With the view of determining if, in the course of development under the conditions found to be most favourable for branching, any evidence of a relation between the branched cells and true mycelial formation could be detected Abbott and Gildersleeve (1903) made various experiments. Cultures of the strains which were found to be most prone to branching were evenly distributed over solidified slightly acid egg albumen mixture in a Petri dish and incubated at 37° C. Impression preparations were made at the end of every two hours. They failed however to detect any suggestion of mycelial formation.

In this connection mention must be made of the following observations. Spirig (1899) states that if cultures of diphtheria bacilli be left for long periods colonies may rarely be found with a chalky appearance. Preparations from such colonies show not only club-shaped bacilli but thread-like and coccus-like forms, which are reproduced on subcultivation. These organisms are non-virulent. Concetti observed (1901) in a mild case of diphtheria organisms which possessed both diphtheria-like and long streptothrix-like forms. In neither instance can contamination with a slow-growing streptothrix be excluded.

Cache's (1901) experiments on this subject are more convincing than those which have just been quoted. A culture of the diphtheria bacillus made on Ouchinsky's mineral solution after some months growth showed a yellowish membrane, which consisted of ramified and interlaced mycelium including refractive granules. Subcultures showed similar growth, but transplantations on agar and gelatin after a few generations showed typical diphtheria bacilli capable of killing guinea-pigs within 40 hours, and of producing toxin.

Spores.

Diphtheria bacilli do not form spores under any known conditions, though at one time it was considered that the polar bodies might in some way be connected with spore formation.

Reaction to Staining Agents.

Diphtheria bacilli take up the basic aniline dyes with great readiness. The staining reagent generally employed for diagnostic work is *Loeffler's methylene blue* solution, which consists of

Saturated solution of methylene blue in alcohol	30 c.c.
Solution of potassium hydrate in distilled water (1—10,000)	100 c.c.

Fixed films may be stained by the application of this solution for five minutes or longer in the cold. They are then washed in water, dried, and mounted in Canada balsam.

Stained by this means diphtheria bacilli show the irregularities of colouration already described, some showing marked segmentation, others a beaded appearance, while others are uniformly stained. In most cultures the majority of the diphtheria bacilli exhibit round or oval bodies clearly distinguishable owing to their dark violet-red colour. Not infrequently these bodies produce distinct swellings in the organisms, and sometimes one seems to occupy the whole of the dilated extremity of a bacillus. These bodies have been termed metachromatic granules, Babes Ernst corpuscles, or polar bodies, by various authors and are also known by several other names. They are usually situated near the ends of the bacilli and are generally surrounded by less darkly stained areas of protoplasm. One or more are, however, not infrequently to be found in the central portions. Throughout this section they are termed polar bodies. Special staining methods have been described, such as Neisser's method, for more clearly differentiating the polar bodies. Their relations to temperature, the age of the culture and its virulence are discussed under the description of Neisser's method.

The appearance of specimens stained by Loeffler's method are best appreciated by reference to Plates VII and VIII.

Cobbett and Phillips (1896) originally described a method by which films made from cultures may be stained and mounted in a dilute solution of Loeffler's methylene blue.

This method was very largely employed in his later work by Cobbett (1901) and the writer has found it to be extremely convenient, easy and reliable for diagnosis. The process consists in making on a cover-glass smears from the colonies on the culture medium. The top of a colony is touched with a platinum needle, and the point is then dipped into water and drawn across the cover-glass. The film thus made is allowed to dry and is then placed film side down on a glass slide on which has been placed a drop of dilute Loeffler's methylene blue (1 : 5). The specimen is almost immediately firmly pressed down on filter-paper with the cover-glass downwards, with the result that the excess of stain is forced from under the cover-glass and absorbed by the filter-paper. Immersion oil is then placed on the cover-glass, and the preparation thus mounted in a small quantity of the staining fluid is examined. If the films are properly prepared no bubbles are found under the cover-glass and the organisms take up the stain and appear to lie in a clear fluid. The segments or more obvious portions of protoplasm are stained dark blue, and the presence of polar bodies is indicated by darkly stained, almost black, granules. (See also, p. 144, and Pl. VI.)

Gram's method. According to some authors diphtheria bacilli retain the stain by Gram's method and according to others they do not. This no doubt may be explained by the fact that the bacillus will not, as a rule, withstand a prolonged action of the decolourising fluid. Under the ordinary conditions of staining by Gram's method the bacillus certainly retains the dye.

Nicolle's method (1895), a modification of the above, has been extensively used by Woodhead (1896) and other observers. The staining fluid consists of 10 c.c. of a saturated solution of gentian violet added to 100 c.c. of a 1 % solution of carbolic acid. The fixed preparation is stained for five seconds and is passed directly into iodine solution (iodine 1 gram, potassium iodide 2 grams, distilled water 200 c.c.), where it remains five seconds. After this it is decolourised by being passed rapidly through a mixture of one volume of acetone with four volumes of absolute alcohol. This treatment removes all unfixed stain almost instantly. The specimen is then dehydrated in xylol and mounted in balsam.

The bacilli also stain well by the *Roux* and *Cladius methods.*

Special Staining Methods for differentiating the Polar Bodies.

The method most commonly used for differentiating the polar bodies is that introduced by *Neisser* (1896) in which two solutions are used.

No. 1.	Methylene blue (Grübler) 1 gram dissolved in alcohol (96 %)	20 c.c.
	Distilled water	950 c.c.
	Glacial acetic acid	50 c.c.
No. 2.	Bismark brown	2 grams.
	Boiling distilled water	1000 c.c.

The films are usually placed in solution No. 1 for one to three seconds, washed in water and placed for three to five seconds in No. 2, but considerably longer periods may be used with advantage especially with solution No. 2. The polar bodies of the bacilli are stained a dark blue, almost black, colour, and the protoplasm brown. Pl. XI, fig. 3 shows the characteristic features of a diphtheria preparation from a young serum culture stained by Neisser's method.

According to Denny (1903) polar granules are first seen as very minute objects after eight hours' growth, but do not become abundant until the cultures have been growing about 15 hours. Neisser (1896) thought that they were characteristic of the diphtheria bacillus provided that cultures on Loeffler's serum were used and examined after 9 to 24 hours' incubation at 34—35° C. Even under these conditions, however, Hofmann's bacillus occasionally shows a few small and irregular polar bodies and some other organisms also exhibit them. The temperature at which the cultures are incubated has a marked influence on the appearance of the polar bodies. Even after 48 hours' growth at 19—21° C. they are very few (Denny, 1903). According to Neisser they are most frequently produced in cultures incubated between 34—36° C., but cultures growing at 37° C. also show them well. When cultures are incubated above this temperature the polar bodies may fail to appear (Neisser) or their appearance may only be delayed (Denny).

PLATE XI.

Fig. 1. Smear preparation from the throat in a case of diphtheria showing *B. diphtheriæ*, a few diplococci and cells. × 1000. (Photograph by Dr James Ritchie.)

Fig. 2. Groups of diphtheria bacilli in a section of membrane stained by Weigert's method. × 600 (Orig.).

Fig. 3. A preparation from a pure culture of *B. diphtheriæ* after 24 hours' incubation stained by Neisser's method. Zeiss $\frac{1}{12}$ Obj. Oc. 4. Drawn with the aid of a camera lucida. (Orig.).

Fig. 4. A preparation from the same culture as the last figure stained by Neisser's method after 48 hours' incubation to show the increase in the number and size of the dark staining areas. Zeiss $\frac{1}{12}$ Obj. Oc. 4. Drawn with the aid of a camera lucida. (Orig.)

In cultures 36 to 48 hours old many of the polar bodies are much enlarged and occupy a large part of the club-like dilations of the cells which have themselves become swollen. Some of these enlarged polar bodies are round, others oval and some elongated. Polar bodies only slightly enlarged and also others of the same size and shape as met with in younger cultures are also very common. In these old cultures the polar bodies appear to be increased in number as well as in size, so that several are often found in one bacillus.

Plate XI, fig. 4 shows the appearances seen in a film stained by Neisser's method from the same culture as Plate XI, fig. 3 after 24 hours' further growth.

Gossage (1896) made numerous experiments with media, to which glycerine had been added, and considered that the polar granules were

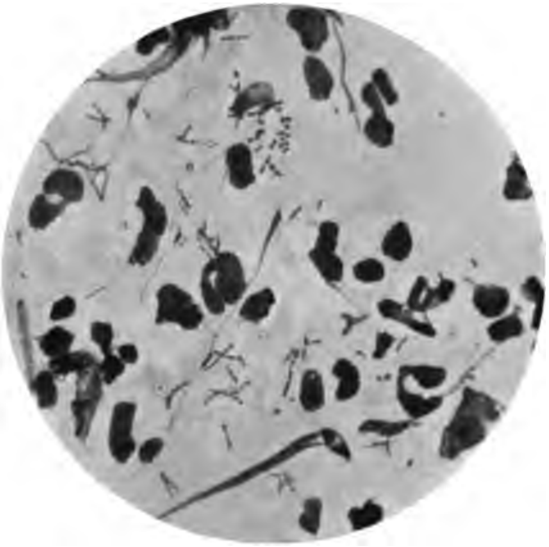


Fig. 1.

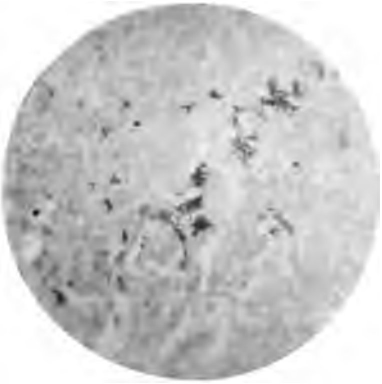


Fig. 2.

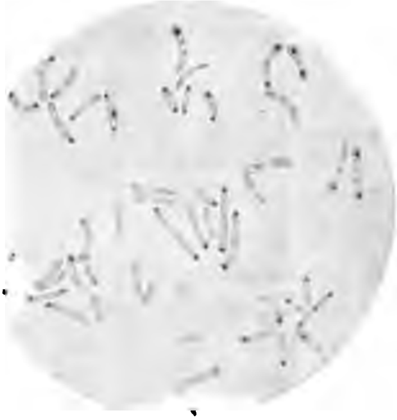


Fig. 3.

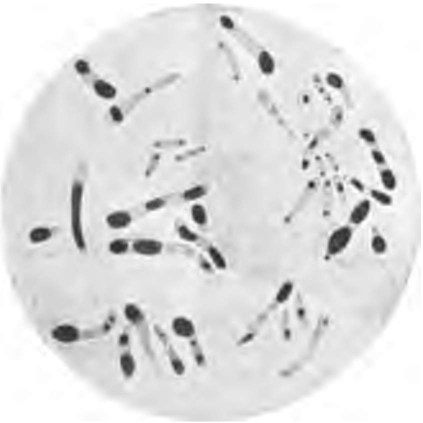


Fig. 4.

rendered more conspicuous by the addition of glycerine to the serum. Kanthack (22. VIII. 1896), however, denied that this was the case.

At one time it was frequently stated that the presence of polar bodies in diphtheria bacilli in cultures examined after 18—24 hours' growth at 36—37° C. indicated that the bacilli were virulent and that the absence of polar bodies indicated a lack of virulence. It has, however, since been proved by the observations of numerous workers that such is not the case. A certain proportion of diphtheria bacilli virulent for guinea-pigs show no polar bodies, and many non-virulent bacilli show well-marked polar bodies.

Reichenbach (1899), for example, found fully virulent bacilli without polar bodies and Park (1900) states that in his hands, with a very large experience, neither Neisser's stain nor its modifications have given any information as to the virulence of the bacilli. Of the 88 virulent diphtheria bacilli discovered by the writer (Graham-Smith, 1904, p. 277) in one epidemic 43·1 % showed well marked, 30·7 % small and poor, and 26·1 % no polar bodies; and of the 25 totally non-virulent diphtheria bacilli 88 % showed well marked, 8 % small, and 4 % no polar bodies. Schumberg (1902) concluded that there was no relation between the gravity of the disease in man and the presence of polar bodies.

Although Neisser's method is the one which has been most commonly used for differentiating the polar bodies, numerous other methods, some of them modifications of it, have been advocated for demonstrating their presence.

In all cases the films are, unless otherwise stated, fixed in the ordinary way and, after staining, washed in water, dried, and mounted in Canada balsam.

Crouch (1896) advocated the use of the following mixture:—

1 % Dahlia	1 part.
1 % Methyl green	5 parts.
Water	4 parts.

The films are placed in the mixture and almost instantly become sufficiently stained. The polar bodies are stained red.

Coles (1899) failed to obtain good results by Neisser's method but stated that constant results could be obtained in the following way:—

(1) The films are stained in Neisser's blue for 10—30 seconds. (2) They are then washed in water and placed in Gram's iodine for 30 seconds, (3) and finally again washed in water and placed in vesuvin (Bismark brown) for 10—30 seconds.

The bacilli are stained brown with blue granules.

Bronstein (1900) first stained with a dahlia solution consisting of dahlia 1 part, alcohol (95 %) 20 parts, and glacial acetic acid 50 parts, for 30 seconds to one minute. After washing with distilled water the films are counterstained for 30 seconds with Bismark brown (1 %). The bacilli are stained a brown colour and the polar bodies red. By this means he obtained 135 positive results out of 136 different specimens.

Cobbett (1901) showed that after mounting and examining films from cultures in dilute Loeffler's methylene blue (1 : 5) the bacilli can be decolourised by running a drop of 5 % acetic acid under the cover-glass. The polar bodies then show up as dark blue granules in the very pale blue bacilli. The writer has used this method in several hundred examinations and found it to be quick, reliable, and easy.

Piorkowski (1901) recommended staining the preparations for 30 seconds to one minute in aqueous methylene blue (warm), decolourising with 1 % acid alcohol, rinsing with water and counterstaining for five seconds with 1 % watery eosin. The protoplasm is stained dark red and the polar bodies very dark blue.

Schauffter (1902) stained films for one minute in the following mixture:

Loeffler's methylene blue	10 c.c.
Pyronin (Grübler) 5 grams dissolved in 10 c.c. of distilled water	1·5 c.c.
Hydrochloric acid (25 %) 3 c.c. in absolute alcohol 97 c.c.	·5 c.c.

The bacilli appear blue and the polar bodies red.

Falières (1902) made use of a stain consisting of

Methylene blue	2·0 grams.
Borax	·5 „
Distilled water	100·0 c.c.
Absolute alcohol	8 drops.

After staining the films are washed and counterstained with vesuvin for 30 seconds. The polar bodies stain very darkly and are sharply defined in the brown bacilli.

Ficker (1902) used methylene blue (Höchst) 1—10,000 to which 2 % of pure lactic acid had been added. He leaves the films in the stain for one minute. As in Cobbett's method the polar bodies are darkly stained and the rest of the bacillus remains almost unstained

De Rovaart (1902) stained the films for 1—1½ minutes in warm

Loeffler's methylene blue, washed in water and counterstained in vesuvin (1 %) for 1—1½ minutes. The bacilli are stained as in Neisser's method.

Peck (1903) stained the films for 3—4 minutes in Loeffler's methylene blue, washed quickly in water and counterstained in ·2 % vesuvin for 30 seconds. The bacilli are stained brown and the polar bodies blue.

Pitfield (1903) made use of three solutions:—

(*a*)	Silver nitrate	5 parts.
	Saturated alcoholic fuchsin	5 „
	Distilled water	3 „
(*b*)	Pyrogallic acid	1 part.
	10 % caustic soda	5 parts.
	Distilled water	10·0 „
(*c*)	Carbol fuchsin	10 drops.
	Distilled water	10 c.c.

The films are first stained in solution (*a*) which is kept warm, then washed and placed in solution (*b*), and finally stained in solution (*c*) for two minutes.

The bacilli are stained red and the polar bodies which take on a blackish colour are sharply defined.

Neisser (1903) has recently made use of a modification of his original method. His solution, No. 1, is retained and a second solution is substituted for the original No. 2.

Solution (2)	Crystal violet (Höchst)	1 c.c.
	Alcohol	10 c.c.
	Distilled water	300 c.c.

Solutions 1 and 2 are mixed in the proportion of two parts of the former to one of the latter, and films are stained in this mixture for one second, washed with chrysoidin (1·300 filtered) for three seconds, and then washed in water.

Andrade (1904) stains in a mixture of Borrel blue 1 c.c. and vesuvin (1 in 100,000) 50 c.c. for five seconds, washes with distilled water and places in Lugol's iodine solution for one minute, again washes in distilled water and decolourises with absolute alcohol till all the blue has come out, which will do so. The films are finally washed in distilled water, and mounted.

Pugh (1905) recommends the use of a mixture consisting of toluidine blue (Grübler) 1 gramme dissolved in 20 c.c. of absolute alcohol added to 1000 c.c. of distilled water and 20 c.c. of glacial acetic acid. The mixture is applied for two minutes. The protoplasm of the bacilli is stained a faint blue and the polar bodies by artificial light a reddish purple.

Higley (1905) has lately described a method for staining smears from membranes, which is only intended for the use of skilled laboratory workers when an immediate diagnosis is desirable. Two solutions are necessary which have to be freshly prepared.

No. 1 consists of five drops of Kühner's carbolic methylene blue in 7 c.c. of tap water, and No. 2 of 10 drops of carbol fuchsin in 7 c.c. of tap water.

No. 1 is applied for five seconds (not longer). The film is then washed, dried with filter-paper, and placed in solution No. 2 for 45 to 60 seconds and again washed. The diphtheria bacilli are stained a dark red or violet. He states that the bacilli are distinguished by their irregular staining, uneven contour, and the presence of blue polar bodies in some of them.

Ljubinsky (1905) made use of a solution consisting of

Pyoktannin (Merck.)	·25 grams.
Acetic acid (5 %)	100·0 c.c.

Films are stained for 30 seconds to one minute, washed and counterstained with 1% vesuvin for 30 seconds. The bacilli are stained violet, the polar bodies a very dark blue, and appear very large and distinct.

Epstein (1906) used the following method. He stains the fixed film in

1. Either 1 % pyronin solution or Loeffler's methylene blue for 30 seconds.
2. Rinses in tap water.
3. Treats with Gram's iodine for 10 seconds.
4. Rinses in tap water, and dries.

No counter stain is required. When pyronin is used the polar bodies are large, dark and brick red, and the bacilli light red. When Loeffler's blue is used the polar bodies are greenish black, and the bacilli greenish.

Loeffler (1907) has recently described a new method which he considers to be the best for demonstrating the polar bodies. Three solutions are used:

A.	Aqueous solution of Borax (2·5 %) and methylene blue (1 %)	4 pts.
	Unna's Polychrome methylene blue (Grübler)	1 pt.
B.	Eosine A. G. extra (Höchst) ·05 % aqueous solution	
C.	Tropaeolin O. O. aqueous solution	5 pts.
	Acetic Acid	·5 pt.
	Water	100 pts.

The film is stained for one minute in a mixture of equal quantities of A and B and then dipped in C and washed with water. The bacilli are pale and the polar bodies dark blue.

Blumenthal and Lipskerow (1905) have recently made numerous comparative experiments on preparations from cultures and membranes with the methods devised by Neisser, Crouch, Bronstein, Coles, Piorkowski, Pitfield, de Rovaart, Falières, Schauffter, Ficker, Peck, and Ljubinsky. They regard Falières' and Ljubinsky's methods as the most satisfactory, both on account of the sharp definition, size, and deep staining of the polar granules, and of the clearness with which the bodies of the bacilli are outlined.

Special Staining for Demonstrating the Capsules.

Boni (1900) recommends the following method for demonstrating the presence of capsules in diphtheria bacilli.

A fluid is prepared consisting of the white of one egg, 50 c.c. of glycerine, and two drops of formalin. The whole is shaken up and filtered. A small drop of this fluid is placed on a cover slip and mixed with diphtheria bacilli from a culture. The preparation is then allowed to dry and stained with Ziehl's carbol fuchsin for 20—30 seconds, rinsed with water, and dried. It is then stained with Loeffler's methylene blue for four to six minutes, rinsed with water, dried and mounted in balsam. The ground is then stained red, and the bacilli blue, with a clear capsule round them. Preparations made in this way certainly show the appearance described by Boni.

Feinberg (1900) stained diphtheria bacilli by Romanowski's stain and various modifications of it. The bacilli stained a bluish colour, and some showed within them two or three red dots, and others large red masses with a clear network in the middle. Feinberg seemed to regard these as nuclei.

The advantages and disadvantages of some of the most commonly used stains in the bacteriological diagnosis of diphtheria will be discussed in Chapter X.

PLATE XII.

Fig. 1. Photograph of a culture of *B. diphtheriae* after 48 hours' growth on alkaline serum. (Orig.)

Fig. 2. Three serum cultures each inoculated with approximately $\frac{1}{100000}$ c.c. of mucous from the throat of a patient suffering from diphtheria. The cultures have been incubated for four days at 37° C. The raised well-defined colonies present in each case are colonies of *B. diphtheriae*. (From Gordon 1903, Pl. VI. fig. 1.)

Fig. 3. Colonies of *B. diphtheriae* after several days' growth on alkaline serum at 37° C. (Orig.)

Fig. 4. Colony of *B. diphtheriae* (type *a*) after 2 days' incubation at 37° C. on agar, highly magnified. (Orig.)

Fig. 5. An agar culture inoculated with a dilution of the nasal discharge of acute coryza and incubated for 4 days at 37° C. The large colonies are formed by a coccus and by Hofmann's bacillus. The minute colonies, in the majority, are colonies of *B. coryzae segmentosus*. (From Gordon, 1903, Pl. VI. fig. 2.)

The diphtheria bacillus is *non-motile and does not possess flagella.*

Cultivation on Artificial Media.

The methods of procuring pure cultures, the advantages and disadvantages of the various media and the differentiations of diphtheria-like organisms are given under the heading of "bacteriological diagnosis." The characters of the growths and the morphology of the bacilli in pure culture are alone dealt with in this section.

Solid media made from serum or serous exudates.

Owing to their selective action in favouring the rapid growth of diphtheria bacilli and retarding that of most other organisms, some of the media made from serum or serous exudates are first described.

Loeffler's serum.

The medium known as Loeffler's serum medium is that which has been most extensively used in the cultivation of diphtheria bacilli, and many observers unhesitatingly state that it is far superior to any other. The medium is made by adding three parts of ox, or horse, or other serum, to one part of neutral broth containing 1 % of grape sugar, 1 % of peptone and ·5 % of salt. The whole is rendered slightly alkaline and the mixture is solidified in sloping tubes in a Koch's sterilizer, and subsequently sterilized in steam at about 90° C. The medium may also be first sterilized at a low temperature,

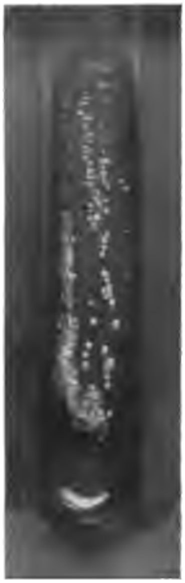


Fig. 1.

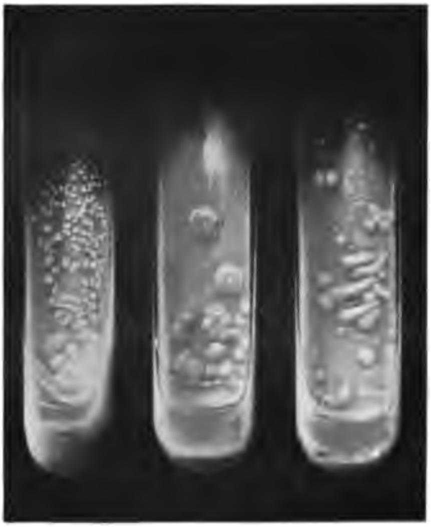


Fig. 2.

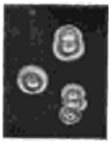
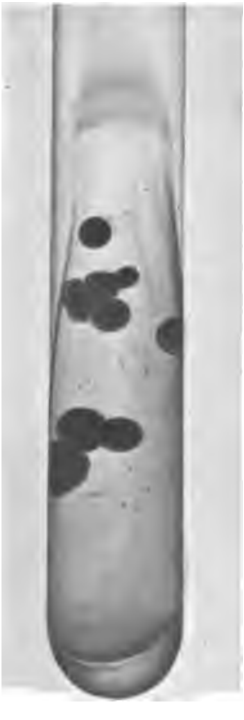


Fig. 3.



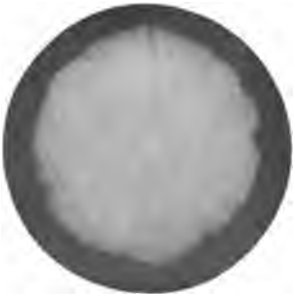


Fig. 4.

Fig. 5.

60—66° C. in steam on six consecutive days, and subsequently solidified. The resulting solid medium is of a whitish colour, opaque in the thicker parts, and gradually becoming clearer until it is almost transparent in the thinner parts of the slope. On this medium diphtheria bacilli grow very rapidly at temperatures between 35—37° C. and minute but distinct colonies may often be seen after 12 hours' growth. After 18—24 hours' incubation diphtheria bacilli produce rounded, elevated, moderately translucent, grayish-white colonies, with a slight yellowish tinge. This yellowish tinge, although very generally present, is slight. The colonies have a moist, shiny, smooth surface, and distinct or only slightly irregular margins. By transmitted light the centre owing to its greater thickness is opaque. When the colonies are few and separate they may grow to a considerable size in this time, but when numerous and close together they remain small. They are almost invariably discrete with distinct intervals between them.

In older cultures the colonies become more flattened, and their central opacity becomes more marked. They lose their moist shiny appearance and become dull, and the surface may become marked, especially round the opaque centre, by very slight irregular, concentric depressions or lines, indicating irregularities in the rate of growth. Not infrequently at this stage radial striation may also be seen, which may extend from the centre to the periphery. The margins no longer remain round but become slightly irregular, and finally crenated. The crenation of the margin is said to be due to the formation of fresh centres of rapid growth, which partially separate themselves from the original colony. This occasionally gives rise to an appearance as if a half, or a portion, of another colony was fused with the first (Pl. XII, fig. 2). Growth is very rapid for some hours after the colonies have become visible but gradually becomes less rapid after 36 hours. On somewhat old serum when the surface is dry, growth is impeded, and may not be rapid at any period.

In *stroke cultures* a gray streak is formed along the needle track. Subsequently growth takes place slowly in a lateral direction, but rapidly along the streak especially towards its lower end. Consequently a thickened raised band is formed down the centre thinning off laterally. The margins are sharply defined and distinctly crenated. At its upper end the streak thins off into isolated colonies with the characters described above. Owing to the fact that in this thinner portion the medium is always drier, these discrete colonies never attain large dimensions. Growth takes place between 22° C. and 40° C., but is most pronounced between 35° C.—37° C. The morphology and staining reactions on this medium are those which have already been described.

Transparent Serum Media.

Lorrain Smith (1894) proposed a serum medium made by adding ·1—·15 % of caustic soda to ox serum and heating the mixture to 120° C. in the autoclave. By this means a transparent jelly was obtained. *Cobbett* (1898) used a modification of this medium, and later worked with a solidified serum medium made in the following way. To every 100 c.c. of ox or horse serum about 1 c.c. of a 10 % solution of caustic soda is added[1]. After thorough mixing 1 % of glucose is added and tubes containing about 5 c.c. of the fluid are solidified and sterilized in a sloping position in steam at a temperature of 87° C.[2] The resulting medium is of a slightly yellowish colour, depending on the amount of colouring matter present in the serum, and quite transparent. The colonies of the diphtheria bacillus on this medium are similar to those produced on Loeffler's serum, but in their early stages are translucent and not so gray. Their subsequent growth is similar, but the yellow tinge is often more marked. (Pl. XII, figs. 1 and 3.)

Kanthack and Stephens' serum agar. In 1896 these authors described a medium made in the following manner.

To every 100 c.c. of serous exudation (pleuritic or ascitic) derived from human subjects 2 c.c. of a 10 % solution of caustic potash is added and subsequently 1·5—2 % of agar (previously soaked in acidulated water and washed). The mixture is then boiled and filtered through a hot funnel and 4—5 % of glycerin is added to it. It is then poured into test tubes and sterilized. The authors say that 2 % of glucose may be added, but state that this does not appear to improve the medium. After sterilization the medium is firm and clear. In making this medium certain precautions are necessary. A small quantity of the exudate should be boiled in a test tube. If it becomes practically solid, owing to the presence of much albumen, it should be diluted to twice its bulk with distilled water. If this is not done the medium becomes gelatinous and useless. The morphology and cultural characters of the bacilli are much the same as on Loeffler's serum.

---

[1] The precise amount of caustic soda solution which it is necessary to add has to be determined by a trial, but according to the writer's experience of many thousand tubes of this medium 1 % is nearly always the quantity necessary. If too much soda is added the medium is very transparent but soft, and if too little, not so transparent, but very firm. The trial is conducted by taking about 5 c.c. of the serum with 1 % of soda solution added and heating in a small steam sterilizer in a sloping position. More serum or more alkali is added according to the result.

[2] Cobbett has shown that this temperature can easily be obtained by passing steam from an autoclave through a Bunsen burner into a closed box containing the tubes in a sloping position. When the requisite temperature has been reached, air is allowed to pass in with the steam by adjusting the collar of the burner to such an extent as to maintain a constant temperature.

Hayward (1895) has suggested a medium made by catching hydrocele fluid in sterile flasks, decanting into test tubes, and sterilizing at 55° C. for three successive days. In the intervals it is kept at 37° C. The tubes are finally slanted and solidified in hot air at 68° C. The bacilli grow in white colonies and retain their usual morphological characters.

White (1895) also employed hydrocele fluid for the preparation of a transparent medium. He added ·07 % of a 10 % solution of caustic soda and sterilized and slanted in the autoclave at 120° C. for 10 minutes.

Horse or ox serum solidified in a Koch's sterilizer and subsequently sterilized in steam at 90° C., or first sterilized at a low temperature and later solidified, forms a medium on which diphtheria bacilli grow well with their usual characteristics.

Several other media containing serum, some of them with complicated methods, have been proposed, but have not been extensively used.

Nutrient agar. Nutrient agar forms a good medium for the diphtheria bacillus, but the growth is not so abundant as on Loeffler's serum. After 24 hours' incubation the colonies are usually still small. The colonies to be found on agar are of two types, though in their growth on other media and their pathogenic action on animals the strains of diphtheria bacilli which give rise to them are indistinguishable. One of these types (*a*) is small, gray or grayish white, almost translucent, rounded, and slightly raised, with a more or less granular surface and dark centre. Sometimes the margins are nearly circular, at other times slightly irregular, or more rarely markedly irregular shading off into a delicate lace-like fringe (Pl. XII, fig. 4). The other type (*b*) of colony, which is less common, is decidedly larger and more luxuriant. It is white, raised, and rounded, with a slightly granular or nearly smooth somewhat moist-looking surface. The culture has the same appearance as that shown on Pl. XII, fig. 1. Growth is often very feeble on agar when the bacilli are freshly obtained from the pseudo-membrane, but becomes more luxuriant after a few transplantations, when the organisms have become accustomed to it. In some cases even after several days' growth the colonies do not become much enlarged, but in others they grow to a considerable size and exhibit the characters of the colonies on old serum cultures. In *stab* cultures growth occurs to the bottom of the line of inoculation as a gray line or as a series of small discrete colonies. Surface growth occurs either in the form of a central white mass with a thin surrounding expansion, or a white smooth dome-shaped mass. In stroke cultures a continuous layer of dull whitish colour is formed, the margins generally showing isolated colonies.

Morphology. In very young agar cultures the bacilli are usually somewhat shorter than on serum, but present otherwise the same morphological characters. In slightly older cultures they are not infrequently very long and very markedly segmented. Polar bodies are frequently absent. Involution forms become common sooner than on serum.

Glycerine agar. Glycerine agar has been very extensively used as a culture medium for diphtheria bacilli. Various proportions of glycerine between 2 % and 8 % have been added by various workers. Gossage (1896) observed the effects of glycerine[1] in glycerinated agar cultures made direct from the throat varying the percentage of glycerine between 6 % and 18 %. He found that as the proportion of glycerine increased the cultures became less impure, owing to the fact that the growth of the ordinary throat organisms was more impeded by the glycerine than that of the diphtheria bacillus. With 6 % of glycerine added the growth was much the same as on serum. With high proportions of glycerine, however, the growth of the diphtheria bacilli was slow. The characters of the colonies on glycerine agar are much the same as upon nutrient agar, the majority being of type (*a*). The morphology of the bacilli on glycerine agar is the same as on nutrient agar.

Joos (1896) recommended a medium made of neutral peptone broth 1000 c.c., albuminate of soda 20 grams, and agar 20 grams, rendered alkaline by the addition of ·15 % of normal caustic soda. He states that on this medium diphtheria bacilli produce good colonies in 15 hours.

Schloffer (1893) used a medium consisting of broth containing 2 % agar 2 parts, and sterile urine 1 part. The bacilli are stated to be rather short and to show very few degeneration forms on this medium.

Capaldi (1896) considered egg agar to be an excellent diagnostic medium, colonies developing in 16 hours. Ordinary agar in tubes is melted and cooled to 45° C. and to each tube 3 or 4 loopfuls of sterile yolk of egg is added and mixed with the agar. The egg agar is allowed to set in a slope.

Gelatin. On gelatin the diphtheria bacillus grows at a temperature of 22° C.—24° C. or a little lower. Growth is slow and the gelatin is never liquefied. The appearance of most of the colonies is the same as that of the colonies on serum, and they undergo the same changes on prolonged cultivation. The colonies soon become dull and lose their shiny appearance. Cobbett and Phillips (1896), who made very careful observations on the appearance of the colonies, describe them as follows: "The colonies of the virulent diphtheria bacilli were of two types, which for the sake of convenience may be termed large and small. The large colonies were for the most part of a whitish colour, with a raised centre and margin, separated by a circular depression. The outline was circular and showed little or no indentation, and only slight radial striation. More rarely the colonies were flatter and thinner, and were definitely divided by a number of radial fissures so that they exactly resembled the daisy-shaped colonies which have been described as occurring on serum. The small colonies were extremely minute. With a lens they were seen to be thin and of the daisy shape." Both kinds may occur in one culture. In *stab* cultures a line of small colonies is formed along the needle track, whilst on the surface a small whitish disc is produced somewhat thicker at the centre.

---

[1] For the effects of glycerine on the formation of polar bodies see page 142.

Morphology. The bacilli show the same morphological and staining characters as those from serum cultures, except that the polar bodies are less prominent and fewer in number.

Potato. Many of the earlier writers state that growth does not occur on potato. On this medium however, especially when the surface is made slightly alkaline, a fairly abundant growth occurs, but can only be seen with difficulty. Growth also occurs on potato steamed without any preliminary treatment. Frequently it is entirely invisible, or may be only indicated by a dry, thin, glaze after some days' incubation. More rarely a perceptible whitish growth occurs, or one with a very slightly yellowish tinge. This character of almost invisible growth separates the diphtheria bacillus from several other bacilli morphologically resembling it.

Microscopical examination after 24 hours' incubation at 37° C. reveals a tolerably abundant growth even when none is perceptible on the surface. Irregular enlarged forms are particularly numerous in potato cultures, and in general the rods are thicker than on other media.

Alkaline bouillon (broth). Ordinary alkaline broth is a good and distinctive medium for the growth of diphtheria bacilli. Usually small whitish granules are formed at the bottom and along the sides of the tube and the medium remains clear without any cloudiness. Rarely a more or less diffuse cloudiness occurs, but the particles generally settle to the bottom in 48 hours or less. Sometimes a slight film forms on the surface of the broth. On shaking the tube this film breaks up and slowly sinks to the bottom. A film or membrane is more apt to develop on the surface of cultures which have been grown for several generations in broth. According to Theobald Smith (1896) the presence of a membrane seems linked to an acidity less than 1·5 % to phenol-phthalein, i.e. an acidity which is neutral or alkaline to litmus. He states that a membrane is, according to his observations, almost proof positive that the acidity is below a certain limit. When only traces of muscle sugar are present a vigorous surface growth begins at once. He is inclined to attribute this to the reaction of the fluid and its action on the bacilli, which he explains from the following observations. According to Chittenden and Gies (*Journ. of Exp. Med.* 1896, I. p. 186) mucin in acid solution sinks to the bottom, but when the fluid is neutralized the mucin swells and rises to the top. The outer envelope of most bacteria, according to Welch, appears to be composed of a substance which behaves like mucin during the process of staining.

Morphology. In the earlier stages of their growth on broth the bacilli are usually slender and less segmented than on other media. But even at an early period irregular and enlarged forms are common, and typical involution forms make their appearance after 3 or 4 days' growth.

The products of the growth of the diphtheria bacillus in broth. In its growth the diphtheria bacillus splits the meat sugar contained in ordinary broth producing acid and thus changes the reaction of the medium, rendering it slightly less alkaline, or even slightly acid. After a variable time the reaction frequently again becomes alkaline.

Roux and Yersin (1888, p. 629) were the first to observe these changes in reaction, and their observations have been confirmed by all subsequent investigators.

Theobald Smith (1893, *Wilder Quarter-century Bk.* p. 224) first pointed out that if diphtheria bacilli be cultivated in broth free from muscle sugar or nearly so, the reaction remains alkaline from the start. Two years later Spronck (1895) independently came to the conclusion that when diphtheria bacilli are grown in broth free from muscle sugar the reaction remains alkaline, when small quantities of sugar are present the reaction becomes acid and later alkaline, but when much sugar is present the broth becomes acid and remains permanently acid. Since that time numerous observers have confirmed the results of these experiments, and have more fully investigated the effects of these changes.

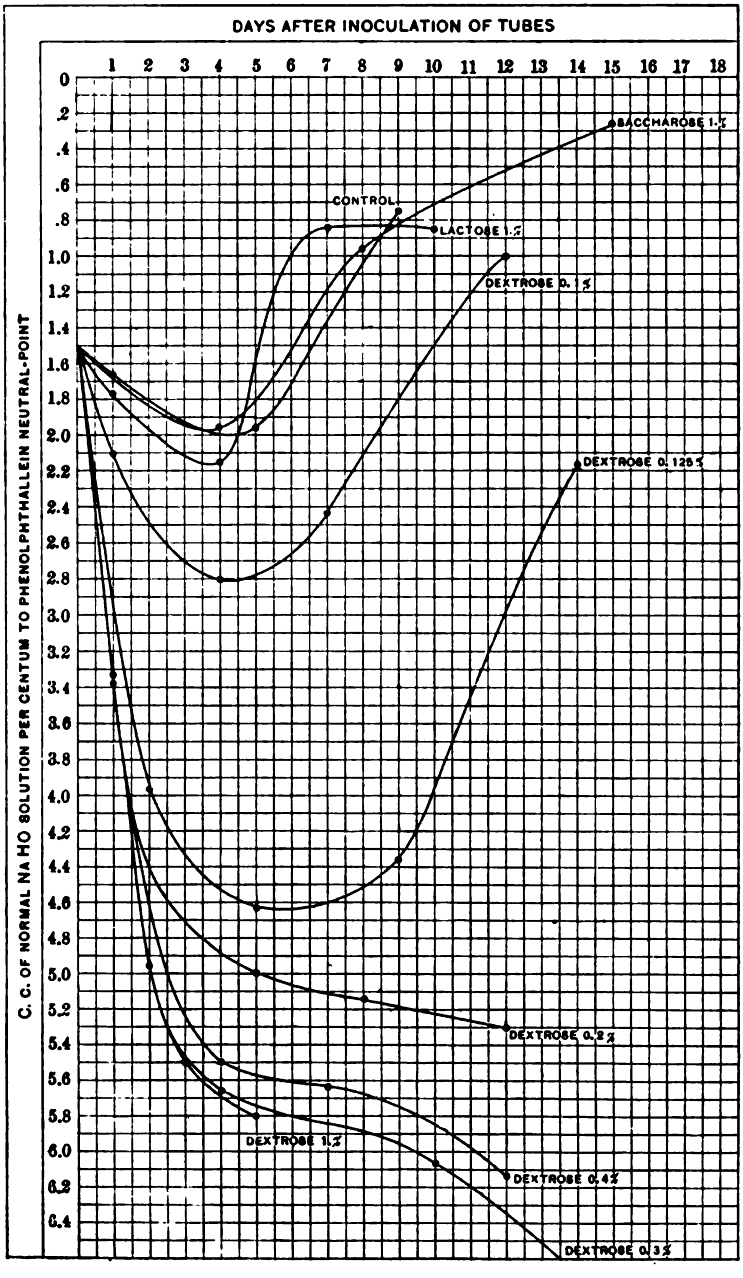
Theobald Smith (1897) tested numerous samples of broth made from beef, and found that the quantities of muscle sugar varied in different samples from the same places at different times of the year. He usually found less present in the winter.

Glucose broth. Theobald Smith (1896) working with broth containing very little sugar, as determined by the action of *B. coli* upon it, investigated the results of the addition of various quantities of glucose (dextrose) to the medium. The broth was sterilized in large test-tubes, which after inoculation with the bacilli were kept in an inclined position in the incubator. He found that the acid reaction promptly formed disappeared after a certain number of days when ·1 to ·125 % of dextrose was used, but when ·2 % was added the cultures became acid and never recovered their alkaline reaction. The degree of acid in c.cs. of normal sodium hydrate required to reduce 100 c.c. of broth to the neutral point of phenol-phthalein is shown in the accompanying chart (p. 156). With percentages of dextrose higher than ·2 % the curves closely follow one another. He also observed that the presence of sugar (dextrose) beyond ·15 % increases the acidity to such a degree that a cessation of the growth becomes manifest. Under the influence of the acid medium the bacilli settle to the bottom, coat the glass with a peculiar thin cloudy deposit, not easily removed by shaking, and the growth thereby comes to a premature close.

Hellström (14. II. 1899) in his investigations recorded the reaction and the number of the bacilli in broth cultures to which varying quantities of glucose had been added. He found that as the acidity increased the growth was retarded and the number became less and less, till finally the culture died, when the acidity reached – 2·5. His figures are given in the following table:—

% of glucose in ordinary broth of initial reaction + 2	20 hours		30 hours		42 hours		60 hours		4 days	
	Reaction	Bacilli per loop								
0 %	+ ·1	1,020	– ·15	29,700	– ·2	10,800	– ·1	976	– ·15	2,984
·1 %	+ ·1	521	– ·4	86,400	– ·7	14,850	– ·9	2,700	– ·9	1,840
·2 %	+ ·1	1,396	– ·6	78,300	– 1·4	12,150	– 1·6	3,860	– 1·7	4,050
·3 %	+ ·1	2,432	– ·8	60,750	– 1·9	5,400	– 2·5	45	– 2·5	61

	5 days		7 days		10 days		21 days	
0 %	– ·2	3,670	– ·2	1,215	– ·2	4,050	– ·2	992
·1 %	– ·9	2,950	– ·9	2,600	– ·9	1,245	– 1·1	1,528
·2 %	– 1·75	3,120	– 1·85	3,340	– 1·9	808	– 2·0	148
·3 %	– 2·45	0	– 2·5	0	– 2·5	0	– 2·5	0


DAYS AFTER INOCULATION OF TUBES
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
C. C. OF NORMAL NA HO SOLUTION PER CENTUM TO PHENOLPHTHALLEIN NEUTRAL-POINT
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.8
6.0
6.2
6.4
SACCHAROSE 1.%
CONTROL
LACTOSE 1.%
DEXTROSE 0.1%
DEXTROSE 0.125%
DEXTROSE 0.2%
DEXTROSE 1.%
DEXTROSE 0.4%
DEXTROSE 0.3%

From his figures it is seen that in ordinary broth, to which no glucose had been added, but little change in reaction occurred, and the bacilli increased in numbers up to 30 hours and subsequently diminished, until after 21 days' growth their number was about the same as at the start. The addition of ·1 % of glucose caused a gradual increase in acidity. The number of bacilli increased up to 30 hours and then gradually diminished. The addition of ·3 % caused a rapid increase in acid and the death of the culture in five days.

Cobbett (1897) confirmed the general conclusions of these observers, but states that contrary to the case of *B. coli* and other bacteria the diphtheria bacillus is not destroyed by the formation of acid in glucose broth, for cultures can be obtained after months.

Almost all workers who have investigated the changes of reaction in broth due to the growth of diphtheria bacilli have used either litmus or phenol-phthalein as indicators. Bronstein and Grünblatt (1902) have recently recommended another method. Their reagent consists of

(1) 2 c.c. of indigocarmine in 100 c.c. of distilled water.
(2) 10 c.c. of acid fuchsin in 100 c.c. of 1 % caustic soda.

Before use two parts of No. 1 are added to one part of No. 2 and 22 parts of water. Some of this mixture is added to the broth before it is placed in test-tubes. Control tubes remain blue, but tubes containing diphtheria bacilli turn a ruby-red colour.

The Action of the Diphtheria Bacillus on Various Sugars and Carbo-hydrates.

Although all investigators are agreed that the diphtheria bacillus acts powerfully on glucose and produces acid, the few observations which have been made on its action on other sugars and carbo-hydrates are not all in agreement. Although little attention has been paid to it, this subject is one which may turn out to be of considerable importance in aiding in the differentiation of the various diphtheria-like organisms from one another and from the diphtheria bacillus.

L. Martin (1898) states that the diphtheria bacillus produces acid from glucose, galactose, laevulose, glycerine and saccharose, but not from lactose, maltose, raffinose, arabinose, dulcite or mannite. The careful experiments of Theobald Smith (1896), however, show that neither lactose nor saccharose is acted on, and most other writers, who have mentioned the subject, agree that acid is not produced from saccharose. Blumenthal disagrees with both these writers in stating that lactose is split up by the diphtheria bacillus. More recently Knapp (1904), working with the serum-water medium of Hiss, has investigated the acid-forming power of 27 races of diphtheria bacilli. He finds that when glucose, maltose, dextrine or mannite are present, the medium is coagulated and made acid, but that lactose and saccharose are not acted on. Hamilton and Horton (1906) found that all races of typical diphtheria bacilli produce acid in media to which dextrine has been added. The writer (Graham-Smith, 1906) has recently investigated the action of 23 races of typical diphtheria bacilli, both virulent and non-virulent, obtained from the throats of persons suffering from diphtheria, convalescents, and contacts, on 1 % solutions of glucose, galactose, laevulose, maltose, dextrine, glycerine, lactose, mannite, and saccharose in the serum-water medium of Hiss and in sugar-free broth. Neutral litmus was used as the indicator. Two sets of experiments were made with Hiss's medium, one series being grown at 37° C. for three days, the other for ten days. The broth cultures were incubated for 48 hours. Acid was never produced in media containing saccharose or mannite. An acid reaction was invariably produced in media containing glucose, galactose, and laevulose. In Hiss's medium (10 days' culture) every specimen produced acid with maltose, and all but one with glycerine, and all but two with dextrine. With lactose six showed a marked acid reaction, ten a slight acid reaction, and four no acid reaction, while in three the reaction was doubtful. Marked coagulation of the medium occurred in almost every tube containing glucose, galactose, laevulose, maltose, and dextrine, and in half the tubes containing glycerine and lactose. In the latter two media many of the tubes were only partly coagulated and in some the medium was only made more viscid or gelatinous. No change was noticed in tubes containing saccharose or mannite. In the series incubated for three days the acid reaction failed to appear in many of the tubes containing lactose and glycerine, and in some containing dextrine and maltose. In the broth cultures acid was always produced with glucose, galactose and dextrine, and sometimes with maltose, lactose and glycerine.

The difference between the series grown on broth and on Hiss's medium is probably due to the fact that some of the bacilli grew poorly on the former, but all grew well on the latter. This corresponds with the well-known facts that recently isolated diphtheria bacilli grow well on media containing serum, but often grow poorly at first in broth. In broth, therefore, the reaction only took place in the presence of those substances on which diphtheria bacilli act readily.

Table showing the action of diphtheria bacilli from different sources on various sugars and carbo-hydrates in the serum-water medium of Hiss and in broth.

			Hiss's medium (10 days' growth)									Broth (48 hours' growth)							
	Source	Virulence	Glucose	Galactose	Laevulose	Maltose	Dextrine	Glycerine	Lactose	Mannite	Saccharose	Glucose	Galactose	Maltose	Dextrine	Glycerine	Lactose	Mannite	Saccharose
1.	Clinical case	virulent	C	C	C	C	C	C	C	–	–	+							
			+	+	+	+	+	+	+	o	o								
2.	Convalescent (26th day)	,,	C	C	•	C	C	c	C	–	–	+	+	o	*	o	o	o	o
			+	+	•	+	+	+	+	o	o								
3.	,, (10th day)	,,	C	C	C	C	C	C	c	–	–	+							
			+	+	+	+	+	+	*	o	o								
4.	Contact	,,	C	C	C	C	C	C	C	–	–	+							
			+	+	+	+	+	+	*	o	o								
5.	,,	,,	C	C	C	C	C	C	C	–	–	+							
			+	+	+	+	+	+	*	o	o								
6.	,,	non-virulent	C	C	C	C	C	C	c	–	–	+							
			+	+	+	+	+	+	*	o	o								
7.	Clinical case	virulent	C	C	C	C	C	c	C	–	–	+	+	o	+	o	o	o	o
			+	+	+	+	+	*	+	o	o								
8.	,, ,,	,,	C	C	C	C	C	c	C	–	–	+	+	o	+	o	o	o	o
			+	+	+	+	+	*	+	o	o								
9.	,, ,,	,,	C	C	C	C	C	c	C	–	–	+	+	+	+	o	*	o	o
			+	+	+	+	+	*	+	o	o								
10.	Contact	non-virulent	C	C	C	C	c	C	c	–	–	+							
			+	+	+	+	+	+	*	o	o								
11.	Convalescent (56th day)	virulent	C	C	C	C	c	C	c	–	–	+							
			+	+	+	+	*	+	*	o	o								
12.	Contact	,,	C	C	•	C	C	c	c	–	–	+	+	*	+	o	o	o	o
			+	+	•	+	+	*	*	o	o								
13.	Clinical case	,,	C	C	C	c	c	C	C	–	–	+	+	*	*	*	o	o	o
			+	+	+	+	*	+	*	o	o								
14.	Contact	,,	C	C	C	C	C	c	c	–	–	+	+	+	*	o	o	o	o
			+	+	+	+	+	*	*	o	o								
15.	Clinical case	,,	C	C	•	C	C	–	C	–	–	+							
			+	+	•	+	+	*	+	o	o								
16.	Contact	non-virulent	C	C	•	C	C	–	–	–	–	+	+	o	+	*	*	o	o
			+	+	•	+	+	*	o	o	o								
17.	,,	,,	C	C	•	C	C	c	–	–	–	+	+	*	+	o	o	o	o
			+	+	•	+	+	*	*	o	o								
18.	Convalescent (35th day)	virulent	C	C	C	c	c	C	g	–	–	+	+	o	+	o	o	o	o
			+	+	+	+	*	+	n	o	o								
19.	Contact	,,	C	C	C	C	c	C	g	–	–	+	*	o	*	o	o	o	o
			+	+	+	+	n	+	n	o	o								
20.	Convalescent (46th day)	,,	C	C	C	C	c	C	–	–	–	+							
			+	+	+	+	+	+	n	o	o								
21.	Clinical case	,,	C	C	C	c	C	c	–	–	–	+							
			+	+	+	+	*	*	o	o	o								
22.	Contact	,,	C	C	C	C	C	C	–	–	–	+							
			+	+	+	+	*	*	o	o	o								
23.	"Sore throat" ("sheath form")	non-virulent	C	g	C	C	–	–	–	–	–	+							
			+	*	+	+	o	o	o	o	o								

C = complete coagulation.
c = partial ,,
g = medium rendered of a gelatinous consistency.

– = no change in appearance.
+ = markedly acid.
* = acid.

n = slightly acid or neutral.
o = no acid formation.
• = not tested.

These tests show that the diphtheria bacillus acts on glucose, galactose, laevulose, maltose, and dextrine, producing an acid reaction, and generally on glycerine and lactose if incubation is continued for 10 days. Mannite and saccharose are not usually acted upon.

More recently the writer has had the opportunity of testing a number of bacilli obtained from outbreaks in several villages in each of which there was probably a single source of infection. Under these conditions it seemed possible that the bacilli isolated from different individuals in the same locality might exhibit similar powers of acting on the various substances mentioned in the serum medium of Hiss. Sixteen strains were isolated from five small outbreaks and compared with 22 bacilli from a larger outbreak. The change produced in the medium in each case was noted daily, and it was found that the degree and rate of coagulation varied as well as the apparent rate of acid formation. In some cases complete coagulation and a high degree of acidity were produced within 24 hours, while in other cases the same result was only reached after several days. All produced a marked acid reaction in the presence of glucose, galactose and maltose, and with one exception (23, slight reaction) in the presence of laevulose. With glycerine 31 produced a marked acid reaction, five (4, 5, 6, 15, 16) a feeble reaction and two (8, 9) no reaction. In no instance was any trace of acid produced with

	Strain	Lactose	Dextrine	Saccharose
Outbreak A	1	0	0	0
	2	0	0	0
,, B	3	0	*	0
	4	0	*	0
	5	0	*	0
	6	0	+	0
,, C	7	0	+	0
	8	0	*	0
	9	0	*	0
	10	0	+	0
,, D	11	+	+	0
	12	+	+	0
	13	+	+	0
,, E	14	•	+	0
	15	+	+	0
	16	+	+	0
Outbreak F	17	0	+	0
	18	+	+	0
	19	*	+	0
	20	0	+	+
	21	0	+	+
	22	0	+	+
	23	0	+	+
	24	0	+	+
	25	0	+	+
	26	*	+	+
	27	*	+	+
	28	*	+	+
	29	*	+	+
	30	*	+	+
	31	+	+	+
	32	+	+	+
	33	+	+	+
	34	+	+	+
	35	+	+	+
	36	+	+	+
	37	+	+	+
	38	+	+	+

mannite. Great differences were noted in the reactions on lactose, dextrine and saccharose, which are given in the preceding table.

The main point of interest is that bacilli from the same locality, and therefore probably derived from one source, usually behaved in the same way. None of those from outbreaks A—E produced any reaction with saccharose, but contrary to previous experience (see p. 159) the majority of strains from F produced a marked acid reaction.

These experiments prove that under suitable conditions all strains of diphtheria bacilli produce acid from glucose, galactose, laevulose, and maltose and the majority from dextrine and glycerine. The action on lactose is very variable and only a few strains act on saccharose. All tests on mannite yielded negative results.

For the action of Hofmann's bacillus and certain diphtheria-like bacilli on these substances see p. 204.

The Effects of Alkalis and Acids on the Growth of the Diphtheria Bacillus in Broth.

(*a*) *The effects of Alkalis.* Cobbett (1897) made a series of sugar free broth tubes (neutral to phenol-phthalein) each holding 5 c.c., containing 2 % of peptone and ·5 % of salt, and added sodium hydrate in quantities corresponding to 10, 20, 30, 40, 50, and 60 c.c. of normal alkali per litre. In tubes containing as much as 30 c.c. of normal alkali per litre the growth was good, in those containing 40 c.c. per litre it was retarded, but it was entirely inhibited by higher degrees of alkalinity. In another series the sodium hydrate was replaced by the alkaline products of the bacilli themselves obtained by distillation. The results were exactly the same.

(*b*) *The effects of Acids.* (Cobbett, 1897.) Three series of similar tubes were made, but containing acid instead of alkali, corresponding to 2·5, 5, 7·5, 10, 12·5, 15, 17·5, and 20 c.c. of acid per litre. In series A the acidity was produced by sulphuric acid, in B by hydrochloric acid, and in C by the products of an old diphtheria culture in glucose broth. The results of these experiments are shown in the following table:

Acidity corresponding to c.c. of acid per litre	Series A		Series B		Series C
	Set 1	Set 2	Set 1	Set 2	
2·5	growth	growth	growth	growth	growth
5·0	,,	,,	,,	,,	,,
7·5	no growth	growth retarded	growth retarded	growth retarded	growth retarded
10·0	,,	trace of growth	,,	,,	,,
12·5	,,	no growth	no growth	,,	no growth
17·5	,,	,,	,,	no growth	,,
20·0	,,	,,	,,	,,	,,

In all cases the growth was retarded by an acidity corresponding to 7·5 c.c. of acid per litre, and it was completely inhibited, except in one instance, by an acidity corresponding to 12·5 c.c.

The Influence of Sugar on Toxin Production.

Among the products formed by the growth of the diphtheria bacillus in broth are toxins, etc. These will be fully dealt with later (Section V), but some observations on this point must be mentioned here. The formation of acid from the muscle sugar in ordinary broth is not only of importance in diagnosing the diphtheria bacillus from other organisms, but has a considerable influence on the production of toxin.

Roux and Yersin (1888, p. 629) were the first to point out that the toxicity of the cultures begins with the alkaline reaction. Theobald Smith (1893) found that the amount of toxin was to some extent inversely proportional to the quantity of the muscle sugar in the broth. Spronck (1895) quotes an experiment which clearly demonstrates the influence of muscle sugar on toxin production. He experimented with two cultures, which in doses of ·2 c.c. after 24 hours' growth, injected subcutaneously, killed guinea-pigs of 250—300 grs. in 24 hours. These were sown on a series of broths made from meat obtained from different sources and incubated at 37° C. He found that the results might be divided into three groups. Group A:—The cultures became acid, and as the acidity increased the development was hindered and the filtrate was not toxic. Group B:—The cultures were luxuriant and the reaction alkaline. After three weeks' growth ·1 c.c. of filtrate killed 500 grm. guinea-pigs in 48 hours. Group C:—Cultures first became acid and later alkaline, but did not produce as much toxin as Group B. These differences he found depended on the amount of sugar in the meat from which the broth was prepared.

These conclusions have been amply confirmed by later investigators and are of great importance in the manufacture of toxins, but are here mentioned on account of their bearing on the pathogenic as opposed to the toxin producing powers of the bacillus.

Sugar-free broth.

From the observations which have just been recorded the necessity for obtaining a broth free from muscle sugar for testing the pathogenic and toxin producing powers becomes apparent. (Cp. section on virulence, p. 173.)

To get rid of the muscle sugar for the preparation of small quantities of broth several means have been adopted, of which the following are the best known:—(1) By allowing the meat to become stale or putrefy before making the broth, thereby making use of the putrefactive organisms to destroy the muscle sugar (Spronck). (2) By adding calcium carbonate to the broth to neutralize the acid as it is formed (Spronck). (3) By inoculating the meat extract with an acid-forming organism, and then after four or five days' proceeding to neutralize, filter, add peptone, and resterilize (Smith). Of these methods Nos. 1 and 3 have been most frequently used (see also Section V).

In testing a sample of broth for the presence or absence of sugar the following method devised by Theobald Smith (1896) is simple and reliable.

Fermentation tubes are filled with the broth, sterilized, and inoculated with *B. coli* or other acid producing bacterium. A simple inspection of the tube from day to day gives sufficient information of the muscle sugar present. If the closed branch of the fermentation tube remains clear, sugar is entirely absent. If it becomes clouded the cloudiness increases in density with the quantity of sugar present. If a little gas appears the quantity of sugar may be still larger in proportion to the gas accumulated. In all cases the open bulb must become clouded or turbid, otherwise the broth is unfit for the multiplication of bacteria.

Ascites broth.

Ascites broth, namely broth to which 25% of ascitic fluid has been added, forms a good culture medium for diphtheria bacilli. (See virulence experiments, p. 175.)

Nicolas and Arloing (1899) compared the growth of diphtheria bacilli on various kinds of broth: (1) ordinary broth, (2) broth made from putrefied meat, (3) ordinary broth to which 10% of human serum had been added, and (4) ordinary broth to which 10% of horse serum had been added. They found that growth was most luxuriant in the order named.

Media made from Various Organs.

Livingwood (1898) made media from the liver, spleen, and suprarenal bodies to determine whether the extracts exerted any marked influence. He found that very little difference was noticeable in the growths, except that the growths on media made from spleen and suprarenal extracts, filtered through porcelain and not heated, were less than those on media made from these organs in the ordinary way.

Cesaris Demel (1899) grew the bacilli on liver broth to which neutral litmus had been added until a violet-amethyst colour was produced. In 24 hours the diphtheria bacillus gave a rosy colour and red deposit, but the Hofmann's bacillus made the broth violet, while the deposit was colourless.

Mayer (1899) made broth from the salivary glands of freshly killed animals (calf, ox, horse, and pig), and media from muscle-juice, urine, gall, aqueous humour, and mucin. He found that on these media slight differences of growth took place, but they were not of great importance.

Glaessner (1900) made numerous experiments to test the efficacy of various substances as substitutes for peptone. The substances used were asparagin, somatose, and nutrose, with and without glycerine, and these were tested against broth made with peptone either alone or combined with glycerine. The bacilli were counted at the start of the experiment, and after 11 and 15 hours' growth. A greater multiplication took place in the tubes without peptone than in those with it.

Milk.

In *milk* prepared in the usual way the diphtheria bacillus grows well, beginning to develop at a comparatively low temperature (20° C.). The milk remains unchanged in appearance and no coagulation occurs, but the medium becomes acid. (Cp. milk as a food substance, p. 172.)

The bacilli multiply in *Litmus whey* at 37° C. and turn the medium acid. This medium has been used instead of glucose broth to ascertain the acid forming power.

Egg media.

Fraenkel (15. IV. 95, p. 349) was the first to recommend cooked egg as a culture medium for the diphtheria bacillus.

"In *cooked and raw eggs* the diphtheria bacilli develop both in the white and the yolk very well; also on *solid egg-albumen* where it sometimes shows branched forms" (Bowhill, 1899, p. 164).

Indol.

It is stated by some writers that the diphtheria bacillus produces indol, while by others this is denied. These differences of opinion probably depend on the methods of testing. Gorini (1893) observed that cholera spirilla failed to give the cholera red reaction when ·5% of dextrose, saccharose, or lactose was present, and Kruse (1894) says that if the broth contains ·25% of sugar the indol reaction no longer appears. Theobald Smith thinks that peptone solution is a bad medium for testing the formation of indol, and considers dextrose-free broth to be better. He found that while in the former the reaction only appeared in 3—6 days, in the latter it appeared in 16 hours, and was very bright. He also observed that in broth containing muscle sugar the reaction was only positive when the sugar had been converted into acid and the latter nearly neutralised. His experiments led him to conclude that the diphtheria bacillus formed no indol. Dzierzgowski and Rekowski (1895) grew diphtheria bacilli in flasks containing 2% peptone in distilled water for six weeks at 36·5° C., and kept control flasks under the same conditions. The distillate showed no free volatile or aromatic acids, or indol, skatol, tyrosin, or cadaverin. Petri (1890) and Kitasato (1889) could not find indol in 24—48 hour cultures, and Lewandowski (1890) was unable to detect it by distilling 8—10 day old cultures and testing the filtrate.

On the other hand Palmirski and Orlowski (1895) studied the filtrates from broth cultures, and state that the indol reaction appears after prolonged cultivation. Peters (quoted by Flügge), Hewlett and Knight (1897), and Hewlett (1900) agree with these observers in thinking that indol is produced after three or four weeks' growth. On further observation, Hewlett (1901) found that the quick reaction which he obtained by the addition of a strong acid and a weak nitrite solution to diphtheria cultures, was not due to indol, which is volatile, but to a non-volatile substance, *skatol-carbolyxic acid.* He isolated this in the following way. The organisms were grown on ordinary broth or peptone water (2—3%) for three or four weeks, and the cultures were then filtered through paper, and evaporated to ½—⅓ of their bulk. The fluid was then acidulated with acetic acid and saturated with ammonium sulphate. This process precipitated the proteoses, etc. which were filtered off, and the filtrate was then evaporated to dryness at 37° C. The dry mass was then ground to a fine powder and repeatedly extracted with boiling alcohol. The alcoholic extracts were mixed, the alcohol distilled off, and the residue dissolved in a small quantity of water, and extracted with ether. The skatol-carbolyxic acid being freely soluble in ether was found in the etherial solution. The tests for skatol-carbolyxic acid are given by Salkowski (*Zeitschr. f. physiol. Chem.* IX. 1885, pp. 8—23) as follows:

(1) Solutions of 1—1000 to 1—10,000 mixed with a few drops of pure nitric acid (sp. gr. 1·2) and then with a few drops of 2% potassium nitrite give a cherry red colour. This is easily soluble in acetic ether and amyl alcohol, but not in ether or chloroform. The solution of acetic ether on the addition of sodium hydrate becomes decolourised, and the soda solution becomes coloured; on acidulating with hydrochloric acid the acetic ether again takes up the colouring matter and the watery layer is decolourised.

(2) Solutions of 1—1000 mixed with an equal volume of hydrochloric acid and a few drops of 1—2 % chloride of lime give a purple red colour.

(3) Solutions of 1—1000 mixed with a few drops of strong hydrochloric acid and two or three drops of weak ferric chloride show a cherry red colour on heating.

Gas Production.

During its growth the diphtheria bacillus does not produce gas under any conditions.

Anaerobic Cultures.

It is generally stated that the diphtheria bacillus grows rather less freely in the absence of oxygen than in aerobic cultures. Hewlett and Knight (1897, p. 15) make the capacity for growth in hydrogen one of the distinguishing features between the diphtheria and the Hofmann's bacillus. They state that the former grows freely, but that the latter shows no growth. Peters, on the other hand, states that it does not grow anaerobically in an atmosphere of hydrogen.

Vitality in Culture.

Under favourable conditions the diphtheria bacillus remains alive in cultures for many months. Nedrigailoff (1901), for example, found that the diphtheria bacillus retained its virulence and vitality when kept on serum in sealed tubes for four years. Loeffler (1890) observed that cultures remained alive on gelatin for 330 days, and Abel (1893) succeeded in obtaining growths from three old dried agar cultures after 213, 212, and 169 days respectively. A fourth culture, however, tested after 163 days, was dead. For the purpose of preserving cultures sugar-free gelatin is a good medium. The tubes should be kept at room temperature, and protected from the light. Kept at 37° C. cultures die rapidly, usually within a few weeks.

Chromogenic Cultures.

It has already been pointed out that more or less coloration of the colonies is fairly common in cultures grown on serum, especially alkalised serum. Many examples occur which show this colour distinctly, especially after a few generations. Hill (1903) observed that six cultures which were kept growing on serum for 10 months showed a distinct increase in the yellow coloration. After a year's subcultivation one was remarkable in its cultural appearance, though typical in morphology and virulence. Cultures on serum showed a bright yellow colour in three days, but on agar after the same time they showed the ordinary whitish colour. The colour was soluble in chloroform, but the pigment dried by evaporation was not soluble in water. Hill also observed feebly red coloured cultures which, when old, became a dirty, or even a dark, brown.

Abbott (1891) recorded a non-pathogenic diphtheria bacillus which gave rise to a coloured growth on potato. This organism, therefore, probably belonged to the group described in Chapter X.

The Influence of Temperature in Relation to the Growth of Diphtheria Bacilli on Artificial Media.

Diphtheria bacilli begin to develop at a temperature between 18—19° C., but growth is then very slow. They grow more rapidly as the temperature is raised, and attain their maximum development at about 35°—37° C. Roux and Yersin (1890) found that young cultures exposed to a temperature of 40° C. died in four days. According to Park (1900), however, development still occurs between 40—41° C., and he states that at this temperature the organisms may retain their virulence for months.

A. Powers of Resistance of Diphtheria Bacilli from Cultures.

(1) *Thermal death point.* According to the earlier observers, Klein (1889), Welch and Abbott (1891), Zarniko (1890), and Roux and Yersin (1890) the thermal death point with 10 minutes' exposure in capillary tubes is 58° C., and this has been confirmed by all the subsequent observers.

(2) *Effects of Cold.* Abel (4. v. 1895) found that cultures protected from the light and left in the open during the winter, when the temperature on some occasions fell as low as – 23° C., kept alive as long as the experiment was carried on; in some cases up to 86 days. Kasansky (1899) was able to confirm these observations, and in one case found that a culture exposed at times to – 25° C. was alive at the end of 118 days. Testi (1902) froze to – 20° C. and heated to 37° C. for 30 minutes alternately (twelve times) emulsions of diphtheria bacilli in water, and found that cultures subsequently made from them were alive and virulent. Ravenel (10. VI. 99) immersed silk threads, impregnated with a suspension of diphtheria bacilli in water, in liquid air (– 190° C.) without drying. Cultures made from the threads after 15 and 30 minutes' exposure gave vigorous growths.

Macfadyen (1. II. 00) later cooled cultures of diphtheria bacilli to the temperature of liquid air (– 190° C.) for 20 hours without effect. Macfadyen and Rowland (5. IV. 00, pp. 339, 488) made further experiments. They exposed emulsions hermetically sealed in fine capillaries to a temperature of −190° C. for seven days, and to −252° C. for 10 hours without any appreciable effect.

(3) *Effects of Drying.* Hill (1902) dried diphtheria bacilli from agar cultures on glass rods, and left them in a box at laboratory temperature in diffuse light, and with slow diffusion of air. Cultures made from these rods showed that the times at which the organisms died varied greatly, some being dead after seven days, and others alive after 20 days.

Put into tabular form his results were as follows:—

No. of rods exposed	20	22	20	22	22	19	99	22
No. of days of drying	1	2	7	7	10	14	14	20
Positive results	20	22	16	13	12	6	9	2
Negative results	—	—	—	3	5	10	77	15
Contaminated	—	—	4	6	5	3	13	5

Abel (1895) dipped silk threads in the condensation water of cultures (three strains) and dried them over acid. Some of these were kept in the open during winter and some in the room. From time to time cultures were made on serum from these threads. Under both conditions the organisms on these threads were found to be alive up to 56 and even 86 days. Their virulence was not lessened.

Deycke (1898) made observations on the length of life of diphtheria bacilli obtained from agar and broth cultures dried on lime wash, oil paint, and glue covering wood and cement.

The following table is a summary of his results:—

Material	Materials smeared with bacilli from agar culture. Final living growth of bacilli obtained in	Materials smeared with broth culture. Final living growth of bacilli obtained in
Oil paint on wood	4 days	24 hours
Lime ,, ,,	9 ,,	3 days
Glue ,, ,,	18 ,,	3 ,,
Oil paint on cement	24 hours	2 days
Lime ,, ,,	10 days	4 ,,
Glue ,, ,,	10 ,,	5 ,,

Reyes (1895) compared the resistance of diphtheria bacilli placed on various materials in a moist and dry condition kept in the dark and in the light.

Diphtheria bacilli placed on	Kept in the dark		Kept in the light	
	In dry condition	In moist condition	In dry condition	In moist condition
Silk are dead in	5 days	8 days	4 days	6 days
Paper ,, ,,	4 ,,	8 ,,	3 ,,	5 ,,
Linen ,, ,,	12 ,,	18 ,,	5 ,,	15 ,,
Mud ,, ,,	100 ,,	120 ,,	74 ,,	120 ,,
Sand ,, ,,	18 ,,	34 ,,	5 ,,	34 ,,

He finds that by rapid drying over sulphuric acid they are killed at the latest in 48 hours.

Golowkow (1895) made experiments of a similar nature. He took pieces of linen, cloth, and satin, and sterilized them at 120—130° C. These were infected by means of broth cultures, and some were left in the dark and others in the light at room temperature. After certain times the pieces were put into broth and cultivated. He found the bacilli dried on cloth were dead after 13 days, and on linen after 16 to 23 days in the dark, and after 20 days in the light. On a certain gray cloth they were alive and capable of growth after 26 days. On unwashed gray linen the bacilli were dead after 24 hours, but on the same material washed they were living after 20 days. The author thinks this is probably due to some chemical substance in the colour or finish. He states that the virulence of the diphtheria bacillus is diminished by drying.

Germano (1897) also came to the conclusion that diphtheria bacilli remain alive a long time on cloth.

The observations which have just been quoted prove that diphtheria bacilli from cultures, without the protection which portions of membrane and saliva can give them, are capable of remaining alive after drying for very considerable periods.

(4) *Effects of Light.* It is well known that diffuse daylight hinders the growth of diphtheria bacilli even when other conditions are favourable, and that it shortens their period of life in cultures. Direct sunlight has a much more powerful bactericidal influence. Gehrke (1899), for example, found that suspensions of the bacilli in clear water were killed by direct sunlight in 2—8 hours, whereas in water yellow with organic material they remained alive longer. He found that agar cultures were killed in six hours, but that broth cultures, one or two days old, were very resistant to its influence. Ledoux (1895), however, states that even broth cultures are killed within a few days. He also found that bacilli dried in thin layers were rapidly killed by direct sunlight.

(5) *Period of life in soil and dust.* Reyes (1895) found that diphtheria bacilli enclosed in dry mud and kept in the dark remained alive for 100 days, in moist mud in the dark for 120 days, in dry mud exposed to light for 74 days, and in moist mud kept in the dark for 120 days. In dry sand kept in the dark they remain alive for 18 days, but in dry sand exposed to the light for only five days. In moist sand, whether kept in the dark or exposed to the light, they

remain alive up to 34 days. Leighton (1901) found that diphtheria bacilli were still capable of producing a growth after being enclosed for 18 days in clay. The clay was kept moist and warm, and studied periodically. Germano (1897) came to the conclusion that diphtheria bacilli can withstand drying in dust for a long period.

(6) *Putrefaction.* Klein (31. v. 1899) made some observations on the bodies of guinea-pigs dead of experimental diphtheria, which tend to show that under certain conditions diphtheria bacilli can resist the changes due to early putrefaction. Some of these bodies were buried in the earth direct, others in sand, and some in wooden coffins. After 14, 21, and 31 days' burial animals buried in each of these ways were exhumed, and cultures made on Kanthack's ascitic fluid medium from the juice of the swollen inguinal glands. All organisms resembling diphtheria bacilli were proved by cultural and animal inoculation tests. After 14 days the bacilli were alive in all cases, but no diphtheria bacilli could be found after 21 and 31 days' burial.

(7) *The effects of Antiseptics.* Owing to the lack of uniformity in the methods used and the time limit adopted by various observers it is difficult to compare the actions of various antiseptics on the diphtheria bacillus. In the following table is given the action of various substances on broth cultures or fresh emulsions of diphtheria bacilli, under either of which conditions the organisms are easily affected by the reagents.

Table showing the action of some common germicides on the diphtheria bacillus in fluid cultures.

Acids—Carbolic 1 : 100	Kills within	10	minutes	(Smith and Somerville, 1905).
Hydrochloric 1 : 25	,,	10	,,	
Nitric 1 : 50	,,	10	,,	
Sulphuric 1 : 10	,,	10	,,	
Alcohol (98—30 $^0/_0$)	,,	1	minute	(Buss, 1905).
Ammonia (gas)	,,	4	hours	(von Rigler, 1893).
Chlorine 1 : 10,000	,,	10	minutes	(Williams, 1895).
Creolin 1 : 2000	,,	10	,,	(Smith and Somerville, 1905).
Cyllin 1 : 800	,,	4	,,	(German, 1905).
Formalin 1 : 100	,,	15	,,	
Lysoform 1 : 100	,,	25	,,	
Mercuric chloride 1 : 5000	,,	10	,,	
Tricresol 1 : 33	,,	10	,,	

From such experiments nothing, however, can be deduced as to the action of these germicides on the bacilli in the dried state, or when enclosed in masses of membrane or mucus. Russ (1905), for example, has shown that 98% alcohol does not affect the vitality of diphtheria bacilli dried on silk threads after an exposure of an hour, but kills the bacilli in emulsions within a minute. 30—60% alcohol on the other hand kills the dried bacilli in five minutes, and the bacilli in emulsions in one minute. Mercuric chloride (1—1000), potassium permanganate (1—20), and carbolic acid (1—20) kill thick layers of cultures in 20 seconds (Bowhill, 1899).

B. Powers of Resistance of Diphtheria Bacilli enclosed in Membrane.

It has long been known that in dried pieces of membrane diphtheria bacilli remain alive for long periods of time. Loeffler (1890) observed living bacilli in fragments of dried membrane after 14 weeks, Park and Beebe (1895) after 17 weeks, and Roux and Yersin (1890) found them still alive in pieces of membrane kept in the dark at room temperature after five months. Many other later observers have confirmed these statements. Even when enclosed within pieces of membrane, the diphtheria bacilli are considerably influenced by the external conditions. The presence of light, moisture, or a high temperature may cause them to die more rapidly than when the conditions are more favourable. Roux and Yersin (1890), for example, found that no living bacilli could be obtained from a dry membrane hung up in the open and exposed to the air, sun, and rain after one month. According to these observers the bacilli dried in membrane can resist a temperature of 98° C. for one hour.

The Behaviour of Diphtheria Bacilli in certain Food Substances.

(*A*) *Water*. The period of the vitality of the diphtheria bacillus in water depends on whether the water is pure or contains nutritive substances, on the treatment it has received, and on the external conditions. According to Montefusco (1896) the diphtheria bacillus remains alive in ordinary water for 20 days, and in sterilized water for 45 days. Seiler and Stoutz (1904) thought that it multiplied in sterilized water up to 9—12 days. In distilled water however, according to the observations of D'Espine and de Marignac (1890), it dies within 24 hours. In strongly polluted water the diphtheria bacillus only remains alive six days (Montefusco). Gehrke (1899) found that the action of sunlight on diphtheria bacilli suspended in water differed according to the degree of pollution of the water. Whereas the bacilli suspended in pure water died in 2—8 hours, they died much more slowly in water yellowish with organic material.

(*B*) ***Milk.*** The most extensive experiments on the growth of diphtheria bacilli in various kinds of milk have been made by Schottelius (1896). He compared the growth and multiplication of diphtheria bacilli in broth and in raw and sterilized milk, both at room temperature and at 37° C. He found that the multiplication of the bacilli was greatest when tested after 6, 24, and 48 hours' growth in raw milk, and greater in broth than in sterilized milk both at 37° C. and at room temperature. In one of his experiments he added 1 c.c. of a broth culture to 20 c.c. of sterilized milk, 20 c.c. of milk taken straight from the udder of the cow, and to 20 c.c. of broth. The actual numbers found per c.c. after growth for six hours were:—

	At room temp.	At 37° C.
In raw milk	21,280,000	50,160,000
In sterilized milk	2,280,000	6,080,000
In broth	7,600,000	18,240,000

Eyre (1904) also showed that the rate of multiplication of diphtheria bacilli in milk, obtained as sterile as possible from the udder of the cow, is very great.

	0 hours	24 hours	48 hours	7 days
Diphtheria bacilli	39	1,170	22,000	19,000,000 per c.c.

The conclusions of Montefusco (1896) and Rubinstein (1904) are, however, not in agreement with these experiments. Montefusco came to the conclusion that sterilized milk was a good medium for diphtheria bacilli, but that no further growth took place in raw milk after three days, while Rubinstein says that in raw milk they die in 24 hours. He states, however, that in prepared butter-milk, as given to children, they remain alive for five to seven days.

Klein (1900), on the other hand, stated that in sterilized milk diphtheria bacilli showed no growth at 37° C. in 14 days, but at 20° C. showed copious growth. He also failed to detect growth in sterilized *cream* at either 37° C. or 20° C.

(*C*) ***Butter.*** According to Montefusco (1896) diphtheria bacilli remain alive for two days in butter, but even after six hours have begun to lose their virulence.

(*D*) Klein (1900) found that diphtheria bacilli did not grow on a sterilized block of *cheese* either at 37° C. or 20° C.

(*E*) *Wines* according to their acidity are more or less noxious to diphtheria bacilli (Montefusco, 1896).

(*F*) On *fresh bread* the bacilli are alive after 24 hours, but are dead after 48 hours. On stale bread they live longer (Montefusco, 1896).

Virulence.

The typical virulent diphtheria bacillus when injected into the subcutaneous tissues of a guinea-pig causes the death of the animal within three or four days with certain characteristic lesions. At the autopsy there is found in the subcutaneous tissue at the site of inoculation a whitish necrotic area which varies in size from about ·5 to 2 cms. in diameter. A more or less extensive, often haemorrhagic, gelatinous oedema surrounds this area. Sometimes the oedematous area is small, at other times it occupies most of the surface of the abdomen, and not infrequently it extends over the whole ventral surface, including the axillae, groins, and neck.

On opening the peritoneal cavity the vessels of the omentum and mesentery are generally found to be somewhat congested. Some congestion of the liver, kidneys, and spleen is also common. The most striking and constant feature is, however, the intense congestion of the vessels of the suprarenal capsules. The colour of these organs is changed from the yellow of their normal condition to pink, red, or a very dark deep red. A small quantity of fluid is sometimes found in the peritoneum.

On opening the thorax a quantity of clear straw-coloured fluid is frequently found in the pleural cavities. The quantity of this fluid may vary between traces which cannot be measured up to 15 c.c. or more. The lungs may be normal or congested.

The histology of these lesions and the rarer conditions are dealt with later (Chapter VII). In 1897 Theobald Smith wrote, "We may, for convenience, regard the disease producing power of diphtheria bacilli as made up of two elements, toxicity and virulence. The former represents the rate of accumulation of toxin in culture fluids, and is easily measured; the virulence, on the other hand, which may be regarded as the behaviour of diphtheria bacilli towards living tissue, is as yet an unknown quantity." The toxin producing power is fully dealt with in another section (Section V), and it is only the virulence with which the present section is concerned. A culture of the diphtheria bacillus is said to be fully virulent when it is capable of killing a half-grown guinea-pig after subcutaneous inoculation within three, or at the outside four, days, with the lesions just described. It has hitherto been generally considered that diphtheria bacilli of all degrees of virulence are to be met with from the fully virulent to the totally non-virulent, which even in large doses produce no effect on guinea-pigs. In fact Welch (1891) summarised his conclusions on this point as follows: "It is to be said that this bacillus, as obtained in pure culture from different cases of diphtheria, varies in virulence, as tested upon animals, to a greater degree than any known pathogenic organism," and Abbott (1902, p. 400) more recently concluded his statements on this subject as follows:—"Under certain circumstances with which we are not acquainted *Bacillus diphtheriae* becomes diminished in virulence, or may lose it entirely, so that it is no longer capable of producing death of susceptible animals, and may cause only a transient local reaction, from which the animal entirely recovers. Sometimes this reaction is so slight as to be overlooked, and again careful search may fail to reveal evidence of any reaction at all. This exhibition of the extremes of its pathogenic properties, viz., death of the animal on the one hand, and only very slight local effects on the other, was at one time thought to indicate the existence of two separate and distinct organisms that were alike in cultural and morphological peculiarities, but which differed in their disease producing power. Further studies on this point have, however, shown that the genuine *Bacillus diphtheriae* may possess almost all grades of virulence, and that absence of, or diminution in, virulence, can hardly serve to distinguish as separate species those varieties which are otherwise alike; moreover, the histological conditions found at the site of inoculation in animals that have not succumbed, but in which only the local reaction has appeared, are in most cases characterised by the same changes that are seen at autopsy in animals in which inoculation has proved fatal." Most writers seem to agree with these opinions, and many observers have proved that the degree of virulence has no relation to the severity or mildness of the disease in the patient from whom the culture was obtained. There can then be but little doubt that variations in virulence are to be found amongst diphtheria bacilli, nevertheless there are many reasons for thinking that intermediate degrees of virulence between full virulence and total lack of virulence are much less common than has been generally supposed. In many of the experiments which are quoted to illustrate these intermediate degrees of virulence the conditions are not uniform.

Some investigators had cultivated the bacilli preparatory to inoculation on ordinary broth containing various quantities of muscle sugar, and some even on broth to which glucose had been added; others have used bacilli from solid cultures emulsified in salt solution, or the water of condensation. The times during which the cultures have been allowed to grow, the dose, and the size of the animals have also all been subject to variation. In fact Theobald Smith (1897) went so far as to say that "the large amount of labour which has been expended in comparing the virulence of diphtheria bacilli from different sources is all but wasted."

Some of these sources of possible error are discussed below. The effect of the presence of sugar in the medium on the toxin production and vitality of the bacilli has already been mentioned (p. 162). Cultures which have become acid frequently produce no result on guinea-pigs, although the strain from which they have been sown is highly virulent. It is therefore of importance that the broth used for cultivating the bacilli previous to injection should be free from sugar.

In most experiments the organisms are grown in broth for 48 hours and certain quantities of the culture are then injected. Most experimenters have not, however, paid any attention to the relative degree of growth of the various strains, although it is well known that many strains, which have been recently separated, grow poorly in broth at first, but after one or two transplantations become accustomed to the medium and grow well.

Cobbett (1901) found that three strains in doses of ·1 c.c. of 48 hour cultures failed to kill, but these cultures were poorly grown, and when injection was repeated a few days later, after the culture had passed through one or more generations in broth and had become accustomed to this medium, death took place within 48 hours. The writer (1904) had a similar experience, but found one culture of diphtheria bacilli which even after several transplantations grew poorly in broth, and only killed animals after 12 days. Williams (1902) also found certain bacilli which immediately after isolation showed very scanty growth in broth, and says that "such broth cultures gave very little reaction in guinea-pigs except in large amounts, though the bacilli themselves when inoculated from serum cultures were decidedly virulent. In ascitic broth however, where they grew rapidly and abundantly, they showed a high degree of toxicity." After ascitic broth was used to test the virulence, all the specifically virulent diphtheria bacilli, about 100 cultures, segmented and granular, were found to be highly pathogenic for guinea-pigs, the largest dose required to produce death being ·2 c.c. and the average ·01 c.c.

Half-grown guinea-pigs (150—350 grms.) are generally employed for the virulence test, the larger animals being somewhat more resistant. It has generally been assumed that all guinea-pigs are about equally susceptible, but Theobald Smith (1897), in estimating the absolute toxicity of culture filtrates, came to the conclusion that animals from some sources are much more susceptible to diphtheria toxin than from others. For several years this writer had been experimenting only upon guinea-pigs reared under his supervision. During this time all animals used exhibited a remarkably uniform susceptibility. Latterly (1905) the guinea-pigs purchased from a dealer had to be used, and it was soon evident that for them the minimal fatal dose was about ½—⅗ of that to which the home-bred guinea-pigs succumbed. In seeking some cause for these irregularities in susceptibility he found that the differences were due to family inheritance, and showed that all individuals from a litter possess the same degree of susceptibility or resistance, and that several litters of the same mother are the same in this respect. Further, he found that the darker animals (black, and black and red) were able to stand 10% more toxin than the white ones. Behring (1898) states that Ehrlich obtained from a dealer a race of guinea-pigs showing relatively great resistance, and Anderson (1906) has recently shown that female guinea-pigs, which have been treated with toxin, in many cases (50%) transmit a considerable degree of immunity to their young.

In experiments relating to virulence the statement is frequently made that the animals have died some considerable period after the injection without any symptoms of paralysis, and death is attributed to the inoculation. Theobald Smith (1897) observed that a certain number of guinea-pigs became emaciated some time after full recovery. They grew literally smaller and moved about the cage with arched backs, and the bones could be felt through the skin. In all such cases after they had been chloroformed the kidneys were found remarkably pale, and in some instances perhaps a little enlarged. He does not believe that this condition is due to the toxin, but that it is peculiar to, or at any rate not uncommon in, the guinea-pig living in confinement, as the disease was found in both young and old untreated guinea-pigs.

The experimental animals must also be kept under good conditions and be free from disease. Valagussa and Ranelletti (1898) attempted to render the conditions of their animal experiments as comparable as possible to the conditions in which the poor live. They kept animals under various conditions of fatigue and hunger, and on a poor and short diet, and in foul air. They found that under the influence of these factors the toxic strength of the diphtheria products was heightened and that the animals succumbed more quickly and with more marked lesions. Previous inoculation with the products of saprophytic and pathogenic organisms also weaken the powers of resistance.

It follows from what has just been stated that in attempting to determine the virulence of various strains of diphtheria bacilli the conditions of the experiments ought to be as equal as possible. Cultures of the bacillus, which have become accustomed to the medium and grow well, incubated at 37° C. in sugar free broth for 48 hours, should be used and injected subcutaneously in small doses. Although broth kept under the influence of light and air undergoes changes, in a series of experiments the results are more likely to be comparable if the same broth is used throughout. The variation in the susceptibility of the animals is probably not a factor of much account in testing the virulence, but should be borne in mind. Half-grown guinea-pigs seem the most suited for these experiments.

Under the conditions just quoted very few series of extended observations have been made.

Cobbett (IV. and X. 1901) in two outbreaks at Cambridge isolated 79 cultures of diphtheria bacilli. Of these 11 were totally non-virulent, and the rest were fully virulent killing in doses of ·1 c.c. within two or three days. He found none with intermediate degrees of virulence.

Williams (1902, p. 103), as already mentioned (see p. 175), found no intermediate degrees of virulence. She says: "After ascitic broth was used to test toxicity, all the specifically virulent diphtheria bacilli,—about one hundred cultures,—segmented and non-segmented varieties, were found to be highly toxic for guinea-pigs. The largest dose of a two to six day culture on ascitic broth which was required to produce death in this animal was one-fiftieth cubic centimeter, the average dose being one-hundredth cubic centimeter."

Graham-Smith (1904) during an epidemic isolated the diphtheria bacilli from 113 out of 117 persons, patients and contacts, who were found to be harbouring them. Of these 87 were fully virulent killing in doses of ·2 c.c., and 25 non-virulent, producing no effect in doses of 2 c.c. One only killed after 12 days, but this strain, even after repeated transferences, grew very poorly in broth. As this is the largest series from an epidemic in one place yet examined in this way the results of the injections and autopsies are given in full (Chapter XII).

Theobald Smith and Walker (1896) used the relative accumulation of toxin in culture, eliminating the bacilli by filtration, in comparing the disease producing power of diphtheria bacilli. In giving their reasons for making use of this method Smith says: "The writer is fully aware of the fact that but an instrument of pathogenic power is here dealt with, and under artificial conditions, since we do not know the nature of the nutritive fluid which the bacilli make use of on mucous membranes, nor as a consequence, whether the toxin production in bouillon is a true index of the production of toxin on mucous membranes. The problem is in fact very complex, as with all infectious diseases, and all we can hope to do at a time is to examine one factor of disease as carefully as possible, while eliminating all the others for the time being. The use of living cultures upon animals is of no service in these experiments because it produces at once three variable factors: (1) the bacilli as potential toxin-producers after injection; (2) the poison of their bedies after destruction; and (3) the toxin preformed in the culture fluid injected."

These observers estimated the toxicity of the culture fluid from 42 races of diphtheria bacilli obtained from different localities under equal conditions. The minimal fatal dose varied from ·036—·12 c.c., that is to say, there was considerable uniformity in the toxin producing power, and they came to the conclusion that this power is not so variable as it has generally been considered. They also investigated in the same way cultures isolated from patients at various times after the disappearance of the membrane. There was amongst these also a considerable uniformity in the production of toxin. Cultures of much greater toxin producing power have been isolated, but some of these at any rate have increased in their capacity for producing toxin by prolonged cultivation.

Park (1900) on the other hand states that "the virulence of diphtheria bacilli from different sources, as measured by their toxin production, varies enormously. Between bacilli which produce a great deal of toxin, and bacilli which apparently produce none, we find all grades of virulence."

Summary of virulence experiments.

The pathogenic properties of the diphtheria bacillus can be tested in two ways (1) by the injection of the sterile products of the organism, or (2) by the injection of living cultures. The former method involves a considerable amount of labour, and has the objection that but an instrument of pathogenic power is dealt with. The latter is the method which has been most frequently used, but many objections have been urged against it: that the conditions are artificial, in that subcutaneous inoculation bears little resemblance to the ordinary mode of infection in man, and that the conditions have varied in almost every series of experiments, and in the individual experiments of each series. Nevertheless, if care be taken to equalise the conditions as far as possible, a correct estimate may be formed of the virulence of the bacilli towards guinea-pigs, and satisfactory evidence is yet lacking to show that races non-virulent to guinea-pigs can produce serious disease in man.

The great majority of workers consider that diphtheria bacilli obtained direct from the throats and noses of *clinical cases*[1] vary in virulence, as ascertained by the subcutaneous inoculation of living cultures, to an extraordinary degree: from fully virulent races which in small doses kill guinea-pigs within three days to races which produce no effect on these animals when injected in large doses. The conditions of most of these experiments have not, however, been uniform.

The results of those experiments which have been made under uniform conditions, i.e. the inoculation of well-grown 48-hour cultures in sugar-free broth or ascitic broth into half-grown guinea-pigs, show that the very great majority of races of diphtheria bacilli are either fully virulent or totally non-virulent for these animals. On further investigation it has usually been found that the latter races have been derived not from clinical cases but from infected contacts. In spite of the latter investigations there can be but little doubt that partially attenuated races do occur, but with much less frequency than is generally supposed.

Classification of diphtheria-like bacilli according to virulence.

Park and Beebe (1895) classified the diphtheroid bacilli commonly met with in the throat and nose according to their virulence and power of producing acid in broth:

---

[1] Experiments on the virulence of diphtheria bacilli derived from convalescent patients and infected contacts are given later (pp. 231, 233).

12—2

[1]I. Bacilli identical in appearance both in culture and under the microscope with the diphtheria bacillus.

(*a*) Pathogenic acid producers = Virulent diphtheria bacilli.

(*b*) Non-pathogenic acid producers = Non-virulent diphtheria bacilli.

II. Bacilli somewhat resembling, but shorter and stouter than, diphtheria bacilli.

Non-pathogenic, non-acid producers = Hofmann's or the pseudo-diphtheria bacillus.

Although this classification does not include the more uncommon forms of diphtheroid bacilli, which have from time to time been encountered, it clearly distinguishes between the forms which are of common occurrence in diphtheria investigations. Had this scheme been more frequently adopted much of the confusion might have been avoided, which has arisen owing to the use of the term pseudo-diphtheria bacillus to denote both the non-virulent diphtheria bacillus and the Hofmann's bacillus.

The Distribution of the Diphtheria bacillus.

I. The occurrence of diphtheria bacilli in notified persons.

Novy (1895) gives a table showing the results of observations by European workers between 1886 and 1895, in which 2846 cases of diphtheria were examined and diphtheria bacilli found 2344 times (82·4 %). European and American observers combined examined 8186 cases finding the specific bacillus in 5943 (72·6 %). French investigators in the Pasteur Institute obtained the bacillus in 701 out of 960 cases (73 %), and during 1894 certain German workers satisfied themselves of the presence of the bacillus in 945 out of 972 cases, giving a percentage of 97·2. The work of Park and Morse showed that out of 5340 suspected cases 67·5 % were true diphtheria.

Woodhead (1896) states that of the 12,172 cases admitted into the Metropolitan Asylums Board Hospitals during 1895–6, and certified as suffering from diphtheria, at least 20 %, or about 3000, offered no bacteriological evidence of diphtherial infection.

Josias and Tollemer (1903) found that of 709 cases admitted into hospital diagnosed as clinical diphtheria, only 580 (81 %) showed bacteriological evidence of the disease.

---

[1] Cobbett's (IV. 1901, p. 244) tabulation of Park and Beebe's classification is shown above.

The following table summarises the observations of the Massachusetts State Board of Health for the nine years ending March 31st, 1905:

Clinical diagnosis	Bacteriological diagnosis			Percentage of true diphtheria
	Positive	Negative	Doubtful	
4113 positive	2555	1504	54	61·6
2340 negative	421	1880	39	18·0
2971 doubtful	982	1942	47	33·0
2031 not given	734	1247	50	36·1

The results of the bacteriological examinations of the 30,000 certified cases quoted above show that bacteriological evidence of diphtheria was present in only 71 %. The experiences of most other observers who have examined smaller numbers of cases are in accord with this.

Some of the diseases clinically simulating diphtheria and occasionally mistaken for the latter are given later (Chapter XI).

II. The distribution of diphtheria bacilli in healthy persons, who have been recently exposed to the disease.

Many of the statistics, which have been compiled and quoted, on the distribution of the diphtheria bacillus in healthy persons have been based on the morphological appearances of the bacilli obtained from cultures alone, without any attempt to isolate and test the suspicious organisms. Very few observers have tested the virulence of even a small proportion of the diphtheria bacilli they have observed in such cultures, but many have assumed that all the morphologically typical bacilli were virulent.

Although it is of the utmost importance to ascertain whether virulent diphtheria bacilli ever occur amongst normal persons, who have not been exposed to diphtheria, and although all observers are agreed that virulent and dangerous diphtheria bacilli occur in the mouths of certain healthy persons, who have contact with the sick, yet not infrequently it is difficult to ascertain whether the persons examined by some investigators have been recently in contact with diphtheria or not.

In the following summary of observations an attempt has been made to classify the various observations, which have been made on healthy persons, most importance being attached to the work of those experimenters, who have tested the organisms they have found, and made careful inquiries as to the possibility of recent contact.

The proportion of "contacts," or persons who have recently been in intimate connection with the disease, who become infected with the diphtheria bacillus to those who do not become infected, is subject to great variation according to the investigations of different observers. To some extent these differences probably depend on the measures taken to promptly isolate the sick, the class of persons examined, and the views of the observer as to the importance of the bacilli which he meets.

Contacts have here been divided into classes according to the closeness of their relationship to the diseased persons:—

(*a*) Family contacts, or members of the family to which the diseased person belongs. (*b*) Persons attending on the sick. (*c*) Persons in close connection with the sick in hospital wards or institutions. (*d*) Scholars of infected schools. (*e*) Contacts not included in the above classification.

(*a*) *Infected families.*

Cobbett (IV. 1901) discovered diphtheria bacilli in all the healthy members of one family. The bacilli from three of them were isolated and found to be virulent (100 %). Park (1892) quotes the case of a family of four persons, all of whom suffered from diphtheria and of whom three died. The bacilli in all cases were virulent (100 %). Scheller (1905) recently examined 284 relatives of diphtheria patients and found diphtheria bacilli in 108 (38 %). Three infected families which he carefully examined contained 7, 6 and 3 healthy individuals, and of these 16 persons 14 were found to be harbouring diphtheria bacilli within a few days of the outbreak of the disease (87·5 %). In these families the distribution of the bacilli was extremely interesting, and one example is given below in detail:

		Date of examination in days from the outbreak of the disease						
Age	Clinical diagnosis	2	22	31	37	46	51	58
7 years	Diphtheria	+	+	+	0	+	0	0
1½ ,,	Healthy	0	0	0	0	0	0	0
2¾ ,,	,,	0	0	0	+	0	0	0
8 ,,	,,	+	+	+	+	0	+	0
9 ,,	,,	+	0	+	+	+	0	0
17 ,,	,,	0	0	0	0	0	+	0
31 ,,	,,	+	0	+	+	0	0	0
39 ,,	,,		+	+	0	0	0	0

+ = diphtheria bacilli present in cultures. 0 = no diphtheria bacilli found in cultures.

Spirig (1899) examined the children of two families numbering four and six respectively. There was one case of diphtheria, and of the remaining nine children six were found to be harbouring diphtheria bacilli (66·6 %). Of these six, five subsequently developed the disease. Williams (1896), in an infected family of five persons, found three with diphtheria bacilli (60 %). Park and Beebe (1894) amongst 48 children in 14 infected families found diphtheria bacilli in 50 %. All the cultures tested were virulent. In other families, however, in which isolation was as perfect as possible only 10 % were found to be infected. Kober (1899) made bacteriological examinations of the throats of 128 persons, relatives of patients, and found diphtheria bacilli in 15 (11·7 %).

In this class of contacts, therefore, the proportion of infected persons varies between 100—50 % in families in which strict isolation is not observed, but may be as low as 10 % under the best conditions.

(b) *Persons attending on the sick.*

Numerous examples are to be found of physicians, nurses, and students becoming infected with diphtheria bacilli from patients, but very few observations show what proportion of these persons becomes infected, and in any case the degree of infection under these conditions must depend greatly on the precautions used.

Richmond and Salter (1898) examined the throats of 129 doctors, nurses, and medical students who had diphtheria patients under their charge, and found diphtheria bacilli in 62 of them (48 %).

Pugh (1902) examined the throats of 56 nurses and found diphtheria bacilli in 7 (12·5 %).

(c) *Persons in close connection with the sick in hospital wards and institutions.*

Lister (1898) in the Shadwell hospital examined 125 children, 69 of whom had nasal discharges. Organisms resembling diphtheria bacilli were found in the noses of 61 of them (48 %). 69 out of 242 cultures taken from the throats of the inmates of an institution known as the Bethany Home (Minnesota Board of Health, 1900) showed diphtheria bacilli at a time when there were three clinical cases (28 %). Müller (1897) systematically examined the children (100) in a general ward in Berlin. Four had diphtheria bacilli when the examination began and six were later admitted with them. The latter were all recent contacts. 14 children acquired the bacilli during their stay (16 %).

He found that infection passed from cot to cot. Soerensen (1898–9) during three years examined the patients in scarlet fever wards. During this period 2290 patients passed through the wards amongst whom 54 harbouring diphtheria bacilli were admitted, and these gave rise to 49 cases of clinical diphtheria and 274 (12·5 %) infected contacts. Graham-Smith (1904) out of 48 patients and nurses examined in a general ward found 5 contacts with virulent diphtheria bacilli and one with non-virulent (12 %). Park and Beebe (1895) found 6 out of 55 children in the New York Foundling Hospital to be harbouring diphtheria bacilli. Of these organisms 5 were virulent. Some cases of diphtheria had from time to time occurred in this institution. (Virulent bacilli 9 %.) Gross (1897) examined weekly the throats and noses of all the children in the Boston City Hospital during a period of six months. 316 children were examined, of whom two suffered from diphtheria. Of the 314 children who showed no clinical manifestations of diphtheria 24 at some period gave cultures of diphtheria bacilli (7·6 %). In the State School for Feeble-minded, Faribault (Minnesota Board of Health, 1900), 3 out of 50 persons examined showed diphtheria bacilli (6 %). In this institution there had been no clinical diphtheria for four months. Chatin and Lesieur (1900) made observations on 75 children in an asylum in which there had been one case of diphtheria. 14 of the children were suffering from sore throats, of whom two had diphtheria bacilli (2·66 %); the remaining 61 were free. Johannessen (1895) found virulent diphtheria bacilli in the throats of 7 out of 38 healthy contacts in a hospital ward.

(*d*) *Scholars of infected schools.*

Crowley and Eurich (1904) examined 93 cultures taken from the throats of the teachers and children of the infant department of a school in which 80 cases of diphtheria had occurred. 42 were found to be infected with diphtheria bacilli (45·1 %). Peck (1901), during an outbreak in a boarding-school, found 31 infected contacts amongst the 100 scholars (31 %). From a school in which several cases had occurred 63 children were examined by Cobbett (x. 1901) and 13 infected contacts found (20 %). Cultures from 10 of these were isolated and tested and 6 turned out to be virulent and 4 non-virulent. In a previous outbreak (iv. 1901) he had examined 650 persons, mostly school children, and discovered diphtheria bacilli in 19. These latter were either children attending the school most affected, or were inmates of houses where there were actual cases. The examination conducted for the State Board of Health of Minnesota (1901) in certain schools resulted in the following findings:—

	School	Children examined	Typical diphtheria bacilli in	
1.	Albert Lee	24	6 (25 %)	Diphtheria prevalent.
2.	Elbow Lake	74	15 (20 %)	Diphtheria prevalent.
3.	Faribault	57	6 (10 %)	Diphtheria had been prevalent.
4.	Owatonna public schools	40	2 (5 %)	One case of diphtheria known in the town.
5.	Mankato	30	1 (3 %)	Cases recently in the school.

Berry and Washbourn (1900) examined bacteriologically 148 girls and teachers with abnormal throats living in a school in which several cases of diphtheria had occurred, and discovered diphtheria bacilli in the throats of 19 (12·1 %). Denny (1900) examined 190 boys in a truant school where there were 10 cases of diphtheria, and found 16 with diphtheria bacilli. Of these 15 lived in one house (9 %). Gabritschewsky (1901) in one school outbreak examined 66 healthy children, and found that 21 of them were harbouring diphtheria bacilli (31·8 %). After another school outbreak he examined 230 scholars on their return to the school, which had been closed for two months. 10 harbouring virulent diphtheria bacilli were discovered (4·3 %). Graham-Smith (1902) in an epidemic at Colchester found 54 (10·4 %) persons out of 519 examined harbouring diphtheria bacilli. All these persons were school children or persons connected with schools. Morphological and cultural methods were relied on, as no tests for pathogenicity could be undertaken there. In 1904 Graham-Smith examined several schools during an outbreak of diphtheria at Cambridge with the following results:—

School	No. of persons examined	Notified persons with diphtheria bacilli			Contacts infected with diphtheria bacilli			
		Virulent		Non-virulent	Virulent		Non-virulent	
St Matthew's School								
Infants (March)	317	13 (4·1 %)	4·3 %	0	12 (3·7 %)	4·1 %	4 (1·2 %)	·9 %
„ (Nov.)	59	6 (10·2 %)		0	4 (6·7 %)		0	
Girls	132	3 (2·2 %)		0	5 (3·8 %)		1 (·7 %)	
Catherine Street School								
Class i	47	0	·6 %	0	0	3·1 %	0	·9 %
ii	103	0		0	1 (·9 %)		0	
iii	64	2 (3·1 %)		0	7 (10·9 %)		1 (1·5 %)	
iv	63	0		0	2 (3·1 %)		2 (3·1 %)	
v	41	0		0	0		0	
Sturton Street School	125	3	2·4 %	0	2	1·6 %	1	
Abbey School	33	0		0	0		1 (3 %)	
New Street School	43	0		0	0		1 (2·3 %)	
Park „ „	20	1 (5 %)		0	0		0	
Ross „ „	47	1 (2·1 %)		0	0		0	

Thomas (1904) during 29 school outbreaks examined 1027 children from infected classes in the public schools, and discovered amongst them 77 with diphtheria bacilli (7·5 %). Fibiger (1897) discovered 10 persons harbouring typical diphtheria bacilli out of 134 in an infected school (7·4 %). All the infected contacts shared the rooms of the diphtheria patients and were as a rule the next neighbours. Loeffler (1894) discovered virulent diphtheria bacilli in the throats of four out of 160 school children, examined at a time when diphtheria was prevalent (2·5 %). Pennington (1907) recently examined by means of cultures 375 apparently normal children from the public schools of Philadelphia. Diphtheria seems to have been prevalent in the city at the time. Typical diphtheria bacilli were found in the throats of 40 (9·3 %). Aaser (1895) during an outbreak in a cavalry regiment examined 89 well persons and found diphtheria bacilli, all of which were virulent, in 17 (19 %). Golowkoff (1898) examined 70 cadets during a small outbreak and found diphtheria bacilli in four (5·7 %).

(*e*) *Contacts not in the above classifications.*

Denny (1900) examined 50 contacts and discovered diphtheria bacilli in 6 (12 %). Meade Bolton (1896) amongst 214 persons who had been previously exposed to diphtheria found the virulent bacilli in 45·5 %, and Chapin (1902) found diphtheria bacilli in the throats of 16 % of persons exposed to the disease. Maude (quoted by Lack, 1899) found diphtheria bacilli in 89 (41 %) out of 214 exposed persons. Goadby (1900) obtained cultures from 586 children of the Poplar Union School in which 21 cases of diphtheria had previously occurred. He found in 190 cases (32·4 %) bacilli morphologically identical with diphtheria bacilli. This observation cannot be included amongst the others on school contacts since virulent diphtheria bacilli were found in the milk, and many of those showing diphtheria bacilli probably acquired them from the milk rather than by contact.

Table showing the results of investigations on the infection of healthy contacts with diphtheria bacilli.

Names of observers	No. of persons examined	No. of infected persons found	Percentage of infected persons	
Infected families.				
Cobbett (1901)	9	9	100	66 %
Park (1892)	4	4	100	
Scheller (1905)	16	14	87·5	
Spirig (1899)	9	6	66·6	
Williams (1896)	5	3	60	
Park and Beebe (1894)	48	24	50	

Table showing the results of investigations on the infection of healthy contacts with diphtheria bacilli (continued).

Names of observers	No. of persons examined	No. of infected persons found	Percentage of infected persons	
Persons attending on the sick.				
Richmond and Salter (1898)	129	62	48	37 %
Pugh (1902)	56	7	12·5	
Relatives of the sick.				
Scheller (1905)	284	108	38	29 %
Kober (1899)	128	15	11·7	
Hospital wards and Institutions.				
Lister (1898)	125	61	48	14 %
Minnesota Board (1900) (Bethany Home)	242	69	28	
Johannessen (1895)	38	7	19·5	
Müller (1897)	90	14	16	
Soerensen (1898–9)	2187	274	12·5	
Graham-Smith (1904)	48	6	12	
Park and Beebe (1895)	55	6	11	
Gross (1897)	314	24	7·6	
Minnesota Board (1900) (Faribault)	50	3	6	
Chatin and Lesieur (1900)	75	2	2·6	
Schools.				
Crowley and Eurich (1904)	93	42	45	8·7 %
Peck (1901)	100	31	31	
Cobbett (x. 1901)	63	13	20	
Aaser (1895)	89	17	19	
Minnesota Board (1900)	225	30	13	
Berry and Washbourn (1900)	148	19	12	
Gabritschewsky (1901)	296	31	10	
Graham-Smith (1902)	519	54	10	
Pennington (1907)	375	40	9	
Denny (1900)	190	16	9	
Thomas (1904)	1027	77	7·5	
Fibiger (1897)	134	10	7·4	
Golowkoff (1898)	70	4	5	
Graham-Smith (1904)	1018	42	4	
Cobbett (iv. 1901)	650	19	3	
Loeffler (1894)	160	4	2·5	
Other Contacts.				
Meade Bolton (1896)	214	97	45·5	36 %
Maude	214	89	41·5	
Denny (1900)	50	6	12·0	
Infection probably through both milk supply and contact.				
Goadby (1900)	586	190	32	
	9881	1453	14·7	

Summary.

From the statistics, which have been collected, it can be seen that the proportion of infected contacts depends to a great extent on their relationship to the diseased persons. Amongst the members of infected families in which no precautions are taken to isolate the sick, the proportion of infected contacts may be very high, whilst in families in which every precaution is taken the proportion may be as low as 10 %. More distant relatives as well as persons attending on the sick may become infected in large numbers.

The observations on the inmates of hospital wards and institutions show that a considerable number of such persons are liable to become infected, when the disease breaks out amongst them. Amongst the scholars of infected schools the proportion of children who acquire diphtheria bacilli by contact is naturally smaller, since the opportunities for infection are not so great. Nevertheless, as some of the observations which are quoted show, a high proportion may become infected if little check is placed on the spread of the disease.

The statistics quoted on close contacts, namely members of infected families, relatives, and attendants, show that amongst such persons 36·6 % are liable to become infected, while the mean infection amongst inmates of hospital wards and institutions is 14 %, and amongst scholars of infected schools 8·7 %.

It must be remembered, however, that in these statistics several factors have not been taken into account, which have a great influence on the results. Amongst these are the regulations which are in force for isolating the sick, as soon as they develop the disease, and for keeping them isolated until the diphtheria bacilli have disappeared from their throats and noses. The number of mild unrecognised cases, which are not isolated and go about spreading infection, also exerts a great influence. Again in hospital wards and schools it is generally found that those most exposed to the sick persons by being placed near them for considerable periods are the individuals amongst whom the proportion of infection is highest, so that when these persons only are examined the proportion of infected contacts amongst these classes is raised.

The investigations on the virulence of diphtheriia bacilli in contacts are given at length in Chapter VI, where it is shown that a very large proportion of these persons harbours fully virulent bacilli. In two classes (*c* and *d*) the inoculation experiments which have been carried out showed that 66 % and 81 % respectively of the strains tested were virulent.

III. *The distribution of diphtheria bacilli amongst healthy persons, who have had no opportunity of acquiring them by contact.*

Throughout the literature on diphtheria statements are frequently to be met with which indicate that the authors consider that virulent diphtheria bacilli may be encountered in a certain proportion of healthy individuals, who have not recently been exposed to persons suffering from the disease, or to infected contacts.

Reliable observations on this point, however, are very few, since many of the investigators have not gone to the trouble of isolating and testing the organisms which they have found, but have based their diagnoses on the morphology of the bacilli in culture; nor have they usually made special inquiries into the possibility of recent contact.

Observations of this kind can only indicate in what proportion of persons organisms, more or less resembling diphtheria bacilli, occur, but no conclusions as to their power of transmitting the disease can be made. Nevertheless some observers have drawn far-reaching conclusions from the results of these experiments, even to the extent of stating that one out of every seven normal children amongst the community harbour diphtheria bacilli in their throats, and are therefore a possible source of danger.

In considering these observations, therefore, those which rely for the most part on the morphology in culture without reference to the possibility of recent contact, are separated from those which are based on a thorough examination of the organisms found, including the test on animals, together with inquiries into the antecedents of the infected persons.

(a) *The following statistics are for the most part based on observations relying on the morphology in culture alone.*

Baurowicz (1895) examined the nasal secretion in 50 persons, 40 of whom were suffering from various diseases, but found no diphtheria bacilli.

Besser (1889) also examined the nasal secretion of 57 normal persons without finding diphtheria bacilli.

Beck (1890) made observations on the throats of 66 healthy children and 64 suffering from various diseases without finding diphtheria bacilli, and Zarniko (1890) examined with the same results 18 throats of normal, and 22 of diseased, persons.

Neumann (1902) found no diphtheria bacilli in the noses of 111 normal persons, but found one amongst 87 persons suffering from non-diphtheritic nasal diseases.

Goadby (1900) examined 100 children in a school in which there had been no clinical cases of diphtheria for two years, and found 18 children harbouring diphtheria bacilli, of whom 14 had unhealthy throats. Diphtheria, however, seems to have been prevalent in the neighbourhood.

Parkes (1903) made cultures from 814 children admitted into the Chelsea Hospital and found diphtheria bacilli in 88, and Hewlett and Murray (1901) on examining 385 children admitted into the Victoria Hospital found diphtheria bacilli in 58.

Park and Beebe (1895) considered that at the time of their examinations virulent diphtheria bacilli were present in 1% of the healthy throats in New York. They remark, however, that diphtheria was rather prevalent at the time, and express the belief that "most of the persons in whose throats they exist have been in direct contact with cases of diphtheria."

The Committee of the Massachusetts Board of Health (1902), in their "Report on diphtheria bacilli in well persons," give tables showing the results of the examination of 4250 well persons, mostly from institutions and schools. These observations are almost entirely confined to the morphology of the bacilli in culture. The results in detail are given in the following table:—

Place examined	No. of persons examined	No. of persons infected	Percentage of persons infected	Remarks	
Ontario	50	0	0	Patients and attendants in general hospital.	
Newton	63	0	0	Women students in Wellesley College.	
Springfield	185	0	0	64 school children, 121 prisoners.	
Providence	927	4	0·43	541 school children, 376 tramps, 10 smallpox patients.	
Waltham	297	2	0·67		
Lowell	250	2	0·8	Cotton-mill hands.	
Washington	221	2	0·9	Mostly hospital patients.	
Boston	892	27	3·0	Adults in prison and pauper institutions.	
Orphan Asylum	65	2	3·0		
Brookline	129	3	2·3	School children	All these persons were more or less exposed to diphtheria.
Willard Hospital	82	3	3·6	,, ,,	
Owatonna	247	13	5·2	,, ,,	
Park Rapids	316	17	5·3	Children and adults	
Red Wing	382	22	5·7	,, ,,	
Old Ladies Home	42	5	11·9	,, ,,	
Bethany Hospital	102	21	20·5	,, ,,	

Amongst these 4250 persons 2·89 % harboured morphologically typical diphtheria bacilli. It can be seen, however, that the last seven sets ought more properly to be placed amongst contacts. Excluding these sets 2955 persons, apparently non-contacts, were examined and 39 (1·3 %) showed diphtheria bacilli in their throats. Of these 39 strains of morphologically typical diphtheria bacilli, 12 were tested on animals, and all proved to be non-virulent. Seventeen strains of uniformly staining bacilli were also tested, and three proved to be virulent, all of which were obtained from Providence. Consequently no morphologically typical *virulent* bacilli were found amongst 2028 of these non-exposed persons from eight different places, whilst amongst the remaining 927 from Providence virulent but atypical diphtheria bacilli were found in 0·3 %.

Even if the persons more or less exposed to diphtheria are included, the proportion of virulent bacilli observed is found to be very small. Altogether 4250 persons, exposed and non-exposed, were examined, and in the throats or noses of 123 morphologically typical diphtheria bacilli were discovered (*A*, *C*, and *D* types of Wesbrook). Of these strains 41 were tested on animals and two (5 %) proved to be virulent (Brookline), and 39 (95 %) non-virulent. Of 30 uniformly staining types 27 were non-virulent and three virulent (Providence). One barred form was also examined and found to be non-virulent. Only 7 % therefore of all the bacilli tested were found to be virulent. If the proportion of virulent forms was the same amongst the strains which were not tested only ·14 % of all these persons harboured virulent diphtheria bacilli.

In considering the results of Hewlett and Murray and of Parkes, the results of the Massachusetts Committee and the conclusions of Pennington (1907) and of Pugh (1902) must be borne in mind. The latter observer considers that "in large centres of population, where diphtheria always exists, diphtheria bacilli are to be found in a not inconsiderable proportion of school children. In the absence both of the evidence of clinical diphtheria and of a history of exposure to that affection, the bacilli are, in the majority of cases, of a non-virulent or saprophytic type and of little hygienic importance; in cases on the other hand, where the clinical supports the bacteriological examination, the bacilli are almost certainly virulent, and therefore dangerous; while in cases where the patient is known to have been exposed to infection the chances are great that the organisms are of the pathogenic variety, and such cases should always be regarded with grave suspicion." The

Massachusetts Committee (1902, p. 21) also came to a similar conclusion. They say "that if a healthy person is found to have Klebs-Loeffler bacilli, and there is no connection traceable between that person and a case of diphtheria, the chances are very much in favour of the bacilli being non-virulent." Pennington (1907), as the result of a considerable number of inoculation experiments, comes to the conclusion "that the organisms found in the throat of a well person are, in the majority of cases, without virulence."

Hewlett and Murray only examined three cultures for virulence, and found that while two were non-virulent the other was only slightly virulent.

The following table shows the prevalence of diphtheria bacilli amongst normal persons and others suffering from non-diphtheritic diseases, as ascertained by investigations in which few inquiries appear to have been instituted as to the possibility of recent contact, and in which the virulence of the organisms found was seldom tested:

Observer	Persons examined	Morphologically typical diphtheria bacilli	Bacilli tested for virulence	Virulent diphtheria bacilli	Non-virulent diphtheria bacilli
Massachusetts Committee[1] (1902)	2955	39	12	0	12
Hewlett and Murray (1901)	385	58	3	1	2
Parkes (1903)	814	88	0	—	—
Goadby (1903)	100	18	0	—	—
Neumann (1902)	198	1	0?	—	—
Baurowicz (1895)	50	0	0	—	—
Beck (1890)	130	0	0	—	—
Zarniko (1890)	40	0	0	—	—
Besser (1889)	57	0	0	—	—
	4729	204 (4·3 %)	15	1	14

This series of observations shows that organisms morphologically resembling diphtheria bacilli in all respects are to be found in the mouths and noses of a small proportion of the normal population. The few inoculation experiments which have been carried out tend to prove, however, that the great majority of these bacilli are non-virulent, and therefore probably incapable of giving rise to diphtheria in man.

---

[1] In the table the more or less exposed persons have been omitted. The virulence tests on those organisms which the Committee did not consider typical have also been omitted.

(*b*) *The following statistics only include those in which the suspicious organisms have been tested and in which careful inquiry was made as to the possibility of recent contact.*

Kober (1899) examined 600 healthy school children from 14 different classes, and discovered diphtheria bacilli in 15. Of these 15 twelve had fairly recently come in contact with the disease, and five of them harboured virulent bacilli. The remaining three, from whom no history of contact was obtainable, showed non-virulent bacilli (non-contacts with virulent bacilli 0 %).

Denny (1900) examined 235 healthy individuals (216 children and 19 adults), a large proportion of the well-to-do classes. He only once, in a school girl, found the diphtheria bacillus. So far as was known the girl had not been in contact with a case of diphtheria, and the bacilli were so few that a pure culture could not be obtained (·4 %).

Cobbett (1901) examined 43 healthy school children, and found no diphtheria bacilli. In some recent investigations, which have not yet been published, this author examined cultures from the throats of 91 boys in a reformatory school, isolating and testing for virulence all the diphtheroid organisms, typical and atypical, which were found. Only in the case of a boy who had just joined the school were diphtheria bacilli found. The other 90 had not been in contact with the disease and no diphtheria bacilli were found in their throats (see also p. 212).

Park and Beebe (1895) examined 275 persons, chiefly hospital patients. Diphtheria bacilli were obtained in pure culture from 26 of them, but 23 cultures proved to be non-virulent. The presence of one of the three virulent examples was accounted for by recent contact, and the other two occurred in adults attending the New York Dispensary at a time when diphtheria was prevalent in the city.

Pugh (1902, p. 296) examined the throats of 415 unselected scarlet fever patients admitted into the North-Eastern Fever Hospital, who showed no clinical signs of diphtheria. In the throats of 17 diphtheria bacilli were found. Of these strains five were inoculated into animals and found to be completely non-virulent, and since the others occurred under the same conditions Pugh assumed that they were non-virulent.

Graham-Smith (1903) made cultivations from the throats of 362 healthy non-contacts, and in one case found non-virulent diphtheria bacilli.

Pennington[1] (1907) examined 125 school children from non-infected schools in Philadelphia. Thirteen harbouring diphtheria bacilli were found. Of these strains 11 (86·4 %) were non-virulent, and two virulent. In the latter the evidence excluding the possibility of contact is not very satisfactory.

In another set of investigations 16 strains were obtained from non-contacts of which 11 (69 %) were non-virulent. As diphtheria was prevalent at the time the pathogenic strains may have been derived by contact.

The following table shows the results of observations on normal throats in which careful inquiries were instituted as to the possibility of recent infection, and in which the majority of the suspicious organisms were isolated and tested for virulence.

Observer	No. of non-exposed persons examined	Persons harbouring virulent diphtheria bacilli	Persons harbouring non-virulent diphtheria bacilli
Park and Beebe (1895)	274	2	23
Kober (1899)	588	0	3
Denny (1900)	235	0	1?
Cobbett (1901)	43	0	0
Cobbett (unpublished)	90	0	0
Pugh (1902)	415	0	17
Graham-Smith (1903)	362	0	1
Pennington (1907)	125	2	11
	2132	4 (·18 %)	56 (2·62 %)

Persons found on inquiry to be recent contacts have been excluded from this table.

Remembering the great difficulty often met with in prosecuting inquiry amongst school children, and the class of persons from whom hospital cases are drawn, and amongst whom these investigations were principally conducted, these figures are very striking, and in the absence of further evidence undoubtedly point to the conclusion that virulent diphtheria bacilli are seldom, if ever, present in the throats of healthy persons who have not recently been in contact with cases of diphtheria or infected contacts.

---

[1] This observer usually inoculated at least two guinea-pigs with bacilli obtained from different cultures from each child, making use of bacilli scraped from the surface of the serum. He gives very full tables relating to the possibility of contact in each case. The figures here given are derived from these tables omitting two or three cases in which the data are insufficient.

The Distribution of Non-virulent Diphtheria Bacilli amongst Contacts and Non-contacts.

In the last section it has been shown that non-virulent diphtheria bacilli are to be found in the throats of about 2·6 % of healthy individuals, who have not been exposed to the disease. The observations of Pugh (1902) and of the Massachusetts Committee (1902) show that they are also to be found in the noses of such persons. The former obtained from the nasal cavities of 414 scarlet fever patients on admission to hospital, who showed no evidence of faucial diphtheria or fibrinous rhinitis, 33 bacilli morphologically indistinguishable from the diphtheria bacillus. Of these six cultures were tested on guinea-pigs and all proved to be non-virulent.

Observations relating to the occurrence of non-virulent diphtheria bacilli amongst contacts are not very numerous. Cobbett (IV. 1901) tested the virulence of several strains of diphtheria bacilli which he obtained from healthy contacts. In one outbreak he found 19 out of 650 persons, who had been more or less exposed to the disease, to be harbouring diphtheria bacilli. Nine cultures were tested for virulence, and three were found to be non-virulent. In another outbreak (X. 1901) he examined 63 children from an infected school, and found diphtheria bacilli in ten. Of these ten strains four were non-virulent. At least seven persons therefore (0·9 %) out of 713 harboured non-virulent diphtheria bacilli.

Amongst 1200 persons, more or less exposed to diphtheria, and who were not harbouring virulent diphtheria bacilli, the writer (Graham-Smith, 1904) found 15 who showed non-virulent organisms, morphologically and culturally indistinguishable from diphtheria bacilli (1·2 %). Pennington (1907) found 19 (5·7 %) non-virulent strains on cultures from the throats of 375 school children, most of whom were attending infected schools. In these three sets of observations nearly 2300 exposed persons were examined, and at least 41 harbouring non-virulent diphtheria bacilli discovered (1·7 %).

It would appear, therefore, that in the mouths of 1—2 % of healthy persons, whether recently exposed to diphtheria or not, non-virulent diphtheria bacilli are to be encountered. These figures lend some support to the view, which has been occasionally advanced, that some at least of the so-called non-virulent diphtheria bacilli belong to a separate saprophytic species, though at present they cannot be distinguished by morphology or culture from true virulent diphtheria bacilli.

The Distribution of Diphtheria Bacilli in the Noses of Healthy Persons.

It is undoubtedly the case that a certain proportion of persons may become infected in their noses and not in their throats. In examinations of contacts conducted on the throat only, such persons would be missed. The observations on this point are not very numerous, and the principal records are tabulated below.

Table showing the proportion of persons infected with morphologically typical diphtheria bacilli in the nose, the throat being free.

Observer	No. of persons examined	Persons infected in the nose only	Percentage	
Contacts. (*Hospital wards and Institutions.*)				
Gross (1897)	314	17	5·3	6·3 %
Minnesota Board (1900)				
Bethany Home	242	19	7·6	
Faribault	50	1	2·0	
Massachusetts Committee (1902)				
Willard State Hospital	82	2	2·4	
Bethany Hospital	102	11	10·7	
Old Ladies Home	42	3	7·1	
Schools.				
Minnesota Board				
Owatonna	40	1	2·5	1·9 %
Mankato	30	0	0	
Albert Lee	24	0	0	
Faribault	57	0	0	
Massachusetts Committee				
Brookline	129	1	0·7	
Owatonna	247	6	2·0	
Red Wing	382	5	1·3	
Park Rapids	316	11	3·5	
Non-Contacts.				
[1] Besser (1889)	57	0	0	1·5 %
[1] Baurowicz (1895)	50	0	0	
[1] Lack (1899)	100	13	13	
[1] Neumann (1902)	198	1	0·5	
Pugh (1902)	414	23	5·5	
Massachusetts Committee				
Ontario	50	0	0	
Newton	63	0	0	
Springfield	185	0	0	
Washington	221	0	0	
Lowell	250	2	0·8	
Waltham	297	2	0·6	
Providence	927	4	0·4	
Boston	892	13	1·5	
Orphan Asylum	65	1	1·5	
	5,826	136	2·33	

---

[1] Simultaneous cultures from the throat were not made in these examinations.

In regard to these observations the virulence experiments of the Massachusetts Committee have already been quoted. Pugh's experiments are of especial interest in that his observations were made on unselected cases of scarlet fever on admission into hospital, who showed no clinical evidence of diphtheria. Three of his 23 strains of bacilli were inoculated into guinea-pigs, and all were found to be non-virulent.

Conclusions on the Distribution of Diphtheria Bacilli.

The various records which have been quoted in the preceding pages show that only about 71 % of the persons notified as suffering from diphtheria show any bacteriological evidence of the disease. The proportion of those healthy persons who become infected with diphtheria bacilli by contact with patients, and yet do not suffer from diphtheria, depends largely on the intimacy of their connection with the patients, varying from a mean of 66 % in members of the family, if the conditions for spreading are favourable, to a mean of 8·7 % in the less closely connected scholars in infected schools. The great majority of the bacilli which have been isolated from these contacts and tested on animals have been found to be virulent (see p. 231).

In the throats and noses of healthy persons, who have had no opportunity of acquiring them by contact, virulent diphtheria bacilli are very rarely found, but bacilli resembling them in morphology, but differing in being totally without virulence for guinea-pigs, are more common (2·6 %). Even amongst recently exposed persons such organisms are to be encountered in a small percentage (1·7 %).

Summary of Chapter IV.

The diphtheria bacillus, as obtained from young cultures on serum, is met with in several morphological types, differing mainly in staining peculiarities, but resembling each other in their general features. Long, medium, and short forms are found. None of these types are absolutely peculiar to the diphtheria bacillus, since organisms indistinguishable in morphology have been reported from various sources. The majority of diphtheria bacilli from young serum cultures show polar bodies. The presence of polar bodies does not indicate virulence, nor does their absence indicate a lack of virulence. Similar polar bodies have been found in several other organisms. So far as the investigations on the subject have been carried the "snapping" type of fission movement is peculiar to the diphtheria group of organisms. Many cultures of diphtheria bacilli show branching forms. Diphtheria bacilli are non-motile, and do not form spores under any known conditions. Cultures of diphtheria bacilli do not show any specific characters by which they can be separated from all other diphtheroid organisms. The most important characters are the production of a marked acid reaction in media to which glucose has been added, an invisible growth on potato, and the formation of a granular deposit in broth. The growths obtained on serum and agar are also helpful in diagnosis. The morphological and cultural characters therefore of the diphtheria bacillus, while of great service in distinguishing it from other diphtheroid organisms, are none of them absolutely characteristic. The diphtheria bacillus does, however, differ from all other known organisms in producing a specific toxin, whose action on animals can be neutralised by antitoxin. The bacilli themselves, or their products, when injected subcutaneously into guinea-pigs give rise to specific lesions. Bacilli, indistinguishable from true virulent diphtheria bacilli, occur, which are entirely non-pathogenic to laboratory animals. They are commonly regarded as diphtheria bacilli which have for some reason lost their virulence, and are termed attenuated or non-virulent diphtheria bacilli. Between the fully virulent and the totally non-virulent diphtheria bacilli races of varying degrees of virulence have been isolated, but the occurrence of such races is probably much rarer than has commonly been supposed.

Diphtheria bacilli in a moist condition are killed by an exposure to a temperature of 58° C. for ten minutes, but in a dried condition, or when enclosed within membrane, they exhibit considerable powers of resistance. They are capable of multiplying very rapidly in raw milk.

Diphtheria bacilli are found in about 71 % of the cases clinically diagnosed as diphtheria, and occur in a varying percentage of persons who have been exposed to the disease, depending on the intimacy of their relation to the sick. Although non-virulent diphtheria bacilli are occasionally found in the throats and noses of persons who have not recently been exposed to the disease, virulent diphtheria bacilli are rarely or ever encountered under such conditions.

Note.—Sholley (15. VI. 07) has recently recorded observations on 1000 children, mostly living in tenement houses, who came for treatment to the New York hospitals. "As far as possible inquiry into immediate and recent exposure was made and noted, and no child suffering from an angina or anything suggesting a sore throat, nasal discharge or laryngitis, was included in the series. Eighteen showed virulent diphtheria bacilli (1·8 %) and 38 non-virulent (3·8 %).

CHAPTER V.

THE PSEUDO-DIPHTHERIA OR HOFMANN'S BACILLUS.

Morphology. Reaction to staining agents. Involution forms. Appearances at different stages of growth. Post-fission movements. Branching forms. Influence of temperature. Cultivation on artificial media, serum, agar, gelatin, potato, broth, milk, litmus whey. Indol production. Virulence. Distribution. Seasonal prevalence. Summary.

The Pseudo-diphtheria or Hofmann's bacillus.

It has been already briefly pointed out that the term pseudo-diphtheria bacillus has been made use of by various writers to include several species of bacilli. Some have included under this term all diphtheria-like bacilli differing in any respect from typical diphtheria bacilli. Others again seem to have restricted it to the non-virulent diphtheria bacillus. Finally, many writers have used it to indicate the short, non-pathogenic, non-acid forming bacillus of Hofmann. In this and the following chapters the term will only be used in the latter sense.

The bacillus of Hofmann, or pseudodiphtheria bacillus, is frequently found in the healthy and diseased buccal and nasal secretions.

The Morphology of Hofmann's bacillus.

A. In *smear* preparations from the buccal and nasal secretions the morphological characters of Hofmann's bacillus are much the same as in preparations made from serum cultures, but the organisms are found lying scattered over the field without any special relation to one another. They are occasionally found enclosed within cells.

B. *In culture. Arrangement.* Preparations made from cultures grown on Loeffler's or other serum media show the bacilli usually arranged in small groups of several individuals generally lying parallel to each other. Similar lines of parallel bacilli are frequently found close to each other, but disposed at various angles. Separate individuals or small clumps without any parallel arrangement are not infrequently seen. According to the experience of the writer, the parallel grouping just described is particularly characteristic of this organism. Chains are never formed (Pl. XV, fig. 2).

Size. This organism is generally much shorter than the common forms of the diphtheria bacillus, but forms as long as medium length diphtheria bacilli are occasionally present even in young cultures. The length of typical examples of Hofmann's bacillus varies between 1—1·5 μ.

Shape. The typical Hofmann's bacillus appears as a short, straight, oval bacillus, with rounded ends, and one median, lightly stained transverse septum. In proportion to its length it is much broader than the diphtheria bacillus, and the widest part, which is about equal to 1/8 of the length, is at the middle. Colonies are not infrequently found in which the bacilli are rather longer, and relatively narrower than those described, but resemble them in all other respects.

A few individuals are occasionally met with even in young colonies which are longer than the ordinary forms and have more than one unstained septum, and may have several. Some colonies have a fair number of such individuals (Cobbett, 1901, p. 239), and more rarely colonies are encountered in which nearly all the bacilli are of this form (Graham-Smith, 1903, p. 230). Some of these forms are clubbed and often slightly curved, but are broader and take the stain more deeply than diphtheria bacilli. The stained segments are very dark and well defined and the septa are narrow and run in all cases transversely across the bacilli (Pl. XV, fig. 1). In subcultures such forms revert to the typical short form of Hofmann's bacillus, and behave like it in all cultural characters[1]. In cultures of typical Hofmann's bacilli over 24 hours old the proportion of segmented and long forms is frequently, but not always, increased. Sometimes after 48 hours' growth the bacilli are even shorter than they were at an earlier period.

Staining of the protoplasm. With Loeffler's methylene blue (full strength or diluted 1 : 5), these bacilli stain darkly and, with the exception of the medium septum, evenly. The septum or, in the case of the long forms, the septa are only very slightly stained in the majority of cases. Sometimes bacilli are found which stain uniformly without a median light septum. When mounted by Cobbett's method (see p. 144) in dilute methylene blue solution, almost entirely unstained specimens, like shadows of bacilli, are always to be seen amongst the darkly stained specimens. In young cultures metachromatic granules (polar bodies) are rarely encountered in any of the forms, but occasionally a bacillus may show one or two very small polar bodies, and very rarely a considerable proportion of the bacilli may do so.

---

[1] On serum media the longer forms are only to be met with in cultures taken direct from the throat, when the morphological characters may possibly be influenced by the presence of other organisms or of mucus. *Young* pure cultures from all such forms show only the oval type.

In cultures 36 or more hours old polar bodies are occasionally found, especially in the enlarged forms. These, however, are seldom as sharply defined as in diphtheria bacilli, and are frequently ovoid or elongated instead of round. Stained by Neisser's and other special methods the bacilli in young cultures show no polar bodies except under the conditions just mentioned. The capsules may be demonstrated by Boni's (1900) method.

Involution Forms. Some old cultures of Hofmann's bacillus show numerous and well marked involution forms, others do not. In some cultures, even after prolonged cultivation in broth or on agar, the majority of the bacilli remain short, though they may show more than one light band, and very few long forms are to be found. In other cultures, however, a large number of long and enlarged bacilli may occur under these conditions. Some of the long forms resemble those described as occasionally occurring on young serum cultures, other giant forms may be very long, thick, curved, clubbed and segmented, others again may be oval or pear-shaped. In fact some old cultures of Hofmann's bacillus so closely resemble old cultures of the diphtheria bacillus as to be scarcely distinguishable.

Morphological appearances of the Hofmann's bacillus at various periods of growth on serum.

Denny (1903) studied cultures on serum tubes incubated at 36° C. by means of stained preparations made after 4, 8, 12, 15, 24, 36, and 48 hours' growth. He found that no changes in shape and size took place up to 36 hours, but sometimes after 48 hours' growth the bacilli appeared even shorter than they were at first (p. 132, Pl. IX, figs. 5, 6).

Post-fission movements.

Hill and Rickards (1903) studied the method of division in Hofmann's bacilli, and stated that "snapping" occurred as in diphtheria bacilli and that the post-fission movements were very similar.

Branching forms.

Abbott and Gildersleeve (1903) studied a number of cultures, but never observed branching even when the method most favourable to its exhibition by genuine diphtheria bacilli was employed, nor have branching forms been described by any other authors.

The influence of temperature in relation to the growth of Hofmann's bacillus on artificial media.

Hofmann's bacillus begins to develop at a lower temperature (18° C.) than the true diphtheria bacillus, and growth is more luxuriant. In other respects the conditions of temperature affecting its growth are the same as in the case of the diphtheria bacillus.

The thermal death point in liquid media is 58° C. for ten minutes.

Cultivation on artificial media.

Hofmann's bacillus is *non-motile, without flagella,* and does *not produce spores* under any known conditions.

Loeffler's serum. Hofmann's bacillus grows very rapidly on Loeffler's serum at 36—37° C. After 18—24 hours' incubation the colonies are small, rounded, elevated, and usually pure milk-white in colour. They have a shiny, moist surface and a distinct margin, and are usually discrete, though in crowded cultures the colonies show some tendency to run together. As compared with those of the diphtheria bacillus, the colonies are much whiter, softer, and larger, and usually flatter. Indentation of the margin or radial fissuring can rarely be seen even with the aid of a simple lens. Growth between 24 and 48 hours is usually much more rapid than that of the diphtheria bacillus, the colonies attaining considerable dimensions during this period. In old cultures similar changes take place in the colonies as in the case of the diphtheria bacillus, they become larger and flatter and lose their shining appearance and concentric rings and radial striae can be seen on the surface. At the same time the margins become irregular, and sometimes even daisy-shaped colonies are produced.

On alkaline serum the colonies in the early stages resemble closely those of the diphtheria bacillus, but are usually larger, and somewhat whiter. At a later period they can usually be distinguished by their whiter colour and larger size. Their general characters on this medium are the same as on Loeffler's serum.

The *morphological characters* on these media have already been described.

Nutrient Agar and Glycerine Agar. At 37° C. Hofmann's bacillus grows more luxuriantly than the diphtheria bacillus on agar, forming in 24—48 hours large white, shining, moist, round, smooth, raised colonies with distinct margins without indentations. The colonies retain these characters during several days' incubation, but gradually lose their moist and shiny appearance, and later become flatter and somewhat depressed in the centre. In crowded cultures there is some tendency for the colonies to coalesce.

In *stab* cultures Hofmann's bacillus produces a more luxurious growth than the diphtheria bacillus both on the surface and along the needle tract.

Morphology. In the earlier stages of growth short oval forms only are found, but as growth progresses segmented forms become fairly common, and after a few days numerous involution forms may be seen.

Gelatin. The colonies, produced after some days' growth on gelatin, are very similar to those formed upon serum; namely whitish, circular colonies, well separated from each other. Their surfaces are uniformly rounded and show little or no tendency to indentation of the margin or radial striation. When grown upon gelatin to which 1 % of glucose has been added they assume a more brilliant white colour, and show a tendency to become irregularly spiked at the margin. (Cobbett and Phillips, 1896.) Liquefaction does not occur.

Hofmann's bacillus grows more luxuriantly on this medium than does the diphtheria bacillus. In *stab* cultures *growth* occurs both on the surface and along the needle tract.

Morphologically the bacilli resemble those from serum cultures.

Potato. On potato a copious but invisible growth occurs like that of the diphtheria bacillus. In cover-glass preparations made from early potato cultures the oval form of the bacillus is found, but in older cultures giant, segmented, occasionally clubbed, forms are not rare.

Broth. In ordinary broth incubated at 37° C. a good growth occurs somewhat similar to that of the diphtheria bacillus, but early diffuse clouding of the medium is more common. The tendency, as with the latter organism, is for the cloud to become gradually deposited as fine granules on the sides and bottom of the test tube. Not infrequently a slight surface film may be formed. The reaction of the broth tends to become more alkaline and acid is never produced.

Morphology. In broth cultures this bacillus more closely resembles the diphtheria bacillus than on other media. Here too the oval bacillus with one unstained septum predominates, but giant forms which closely resemble diphtheria bacilli are generally present also, and may be numerous. They are many times the length of the oval bacillus, often clubbed, and are rather thicker than the diphtheria bacillus.

In *glucose broth* the growth is similar to that produced in ordinary broth, but the clouding is often more marked. Neither gas nor acid is ever produced, and in fact there seems to be even an increase in alkalinity.

Sugars and carbohydrates. Hofmann's bacillus does not produce acid even after prolonged growth in broth or Hiss's medium containing glucose, lactose, maltose, galactose, saccharose, laevulose, dextrine, mannite, or glycerine. (Knapp 1904, Benham 1906, Hamilton and Horton 1906, Graham-Smith 1906.)

In *milk* prepared as a culture medium a good growth occurs. The medium does not become acid, nor is it coagulated. Abundant growth occurs in *litmus whey*, but the medium remains alkaline.

Indol. According to Hewlett (1901) old cultures of the Hofmann's bacillus, like those of the diphtheria bacillus, produce skatol-carbolyxic acid, which has been mistaken for indol (see p. 164).

Anaerobic cultures. According to Hewlett and Knight (1897, p. 14) Hofmann's bacillus does not grow anaerobically in hydrogen. Most other writers, who mention the subject, state that growth occurs under the ordinary conditions of anaerobic cultures.

Powers of resistance.

No experiments appear to have been made on the powers of resistance of Hofmann's bacillus to drying, cold, putrefaction, sunlight or disinfectants, or on its capacity for growth on various food substances.

Virulence.

Towards the ordinary laboratory animals, guinea-pigs, rabbits, rats, etc., Hofmann's bacillus is not pathogenic, when injected subcutaneously or intraperitoneally even in large doses. With very large doses a slight and transient oedema may be the result. According to some observers, however (Williams, 1902), guinea-pigs may succumb to the intraperitoneal injection of enormous doses.

Some writers have asserted that this bacillus is pathogenic for small birds of the finch and bunting families. (For an account of these experiments see p. 220.)

The possibility of causing Hofmann's bacillus to become virulent to guinea-pigs in small doses, its action on small birds, its power of producing toxoids in culture, and its agglutination by the sera of animals immunised to the diphtheria bacillus, are questions which are intimately bound up with its relationship to the diphtheria bacillus, and are discussed in the next chapter.

The distribution of Hofmann's bacillus.

Nearly all observers are agreed that the Hofmann's bacillus is to be met with in greater or lesser numbers in the throats and noses of persons recovering from diphtheria, and also of healthy persons. While some consider that they are more frequently discovered in the former class and contacts, others think that they are as common in healthy non-contacts, as in convalescents and contacts. The statistics on the subject are, however, extremely conflicting, partly owing to diverse opinions as to which organisms should be considered as pseudo-diphtheria or Hofmann's bacilli, and partly to the different methods which have been adopted. Some investigators on the one hand have founded their impressions on observations dealing with certain limited classes of the community only, and on the other hand it is not uncommon to find statistics based on experiments which include without distinction members of the following classes: adults and children, non-contacts and contacts, healthy persons and those suffering from diseases of the nose and throat, persons chosen at random and those living under peculiar conditions in schools, institutions, asylums, and hospital wards. Again in very few cases has any attempt been made to separate persons belonging to the well-to-do classes from those belonging to the poorer classes.

In the first of the following tables a considerable number of observations have been brought together, regardless of any of the above considerations, showing the percentage of healthy persons found to be infected in either the throat or nose, or both, with Hofmann's bacillus. There is also added the percentage in each case of persons infected with diphtheria bacilli. The table shows that at least 32% of all these persons were infected with Hofmann's bacillus, and that the number of those infected with this organism bears no relation to the number infected with the diphtheria bacillus.

Table (A) showing the total infection with Hofmann's bacillus in the throat, or nose, or both, of contacts and non-contacts, adults and children.

American observers	Number examined	No. infected with Hofmann's bacillus	Percentage infected with Hofmann's bacillus	Percentage infected with the diphtheria bacillus
Park and Beebe (1895)	330	27	8·1	9·7
Minnesota Board (1900)	443	128	28·8	20·1
Massachusetts Committee (1902)[1]				
(1) In the East	3096	562	18·1	1·5
(2) In Minnesota	1154	510	44·2	6·03
	5023	1227	24·4	
European observers				
Neumann (1902)	206	200	97·0	0
Pakes (1900)	441	428	97·0	0
Jacob (1905)	113	77	68	5·3
Hutchens (1906)	87	49	57	
Pugh (1902)	420	234	55·7	15·9
Lack (1899)	100	52	52	13·0
Graham-Smith (1902–4)	2878	1346	46·7	7·2
Cobbett (unpublished)	90	39	43·3	
Roux and Yersin (1890)	123	49	39	
Cobbett (1904)[2]	1724	673	39	
Chatin and Lesieur (1900)	75	22	30	2·6
Hasslauer (1902)	82	23	28	0
Thomas (1904)	1027	277	27	7·5
Hewlett and Murray (1901)	385	92	23·9	15
Berry and Washbourn (1900)	142	33	23	11·5
Parkes (1903)	814	162	19·9	10·9
Golowkoff (1898)	70	8	11·5	5·7
Symes (1895)	82	12	14·6	14·6
Fibiger (1895)	135	15	11·1	12
Stein (1900)	86	7	8·1	0
Sellner (1897)	103	7	6·8	0
Escherich (1895)	320	13	4·0	4
Garratt and Washbourn (1899)	666	21	3·2	
Hewlett and Thomson (1895)	103	0	0	0
Peters (1896)	25	0	0	0
Baurowicz (1895)	40	0	0	0
	10337	3839	36·9	
Total	15360	5066	32·9	

---

[1] Only type D^2 counted. Examples of Hofmann's bacillus were probably included under types E^2 and G^2, of which many occurred. If the latter types are all included the total infection in American observations is 38·9 %.

[2] Quoted by Graham-Smith (1903, p. 249).

In the following tables (*B* and *C*) an attempt has been made to subdivide the figures given in the previous table for the purpose of gaining a more minute estimate of the numbers of persons infected with Hofmann's bacillus under various conditions.

Table (B) showing throat infection amongst healthy persons with Hofmann's bacillus.

American observers	No. of persons examined	No. infected with Hofmann's bacillus	Percentage infected	
Adult non-contacts.				
Massachusetts Committee (1902)[1]				
Ontario	50	3	6·0	5·5 °/₀
Boston	892	45	5·0	
Lowell	250	0	0	
Springfield[2]	185	15	8·1	
Park and Beebe (1895)	257	27	10·5	
Adult contacts.				
(M. C.) Willard State Hospital	82	0	0	
Children non-contacts.				
(M. C.) Washington	221	1	0·4	0·9 °/₀
,, Providence[3]	927	10	1·0	
Park and Beebe (1895)	18	0	0	
Children contacts.				
(M. C.) Minnesota	1154	168	14·5	13·2 °/₀
,, Brookline	129	15	11·6	
Minnesota Board of Health (1900)[4]	392	39	9·9	
	4557	323	7·0	
European observers				
Adult non-contacts.				
Escherich (1893)	320	13	4	
Adult contacts.				
Golowkoff (1898)	95	14	14·7	12·6 °/₀
Fibiger (1895)	135	15	11·1	
Children non-contacts.				
Hutchens (1906)	87	49	57	24·1 °/₀
Roux and Yersin (1890)	59	26	44	
Cobbett (unpublished. See p. 212)	90	39	43·3	
Beck (1890)	107	36	33·6	
Hewlett and Murray (1901)	385	92	23·9	
Parkes (1903)	725	162	22·3	
Pugh (1902)	420	67	15·9	

[1] Only type D_2 included.

[2] Springfield total made up of 121 male prisoners and 64 school children.

[3] Providence total made up of 386 adults and 541 school children.

[4] Total at Albert Lee, Faribault, Owatonna, and Mankato schools, and the Bethany Home.

M. C. = Massachusetts Committee (1902).

Table (B) showing throat infection amongst healthy persons with Hofmann's bacillus (continued).

European observers	No. of persons examined	No. infected with Hofmann's bacillus	Percentage infected	
Children contacts.				
Cobbett (1904)[1]	1724	673	39	31·0 %
Roux and Yersin (1890)	45	15	33	
Chatin and Lesieur (1900)	75	22	30	
Berry and Washbourn (1900)	142	33	23	
Thomas (1904)	1027	277	27	
Goadby (1900)	586	99	16·9	
	6020	1632	26·8	
	10570	1955	18·2	

[1] Quoted by Graham-Smith (1904).

Table (C) showing nose infection with Hofmann's bacillus amongst healthy persons.

	No. of persons examined	No. infected with Hofmann's bacillus	Percentage infected	
Adult non-contacts.				
Massachusetts Committee (1902)				
Ontario	50	7	14·0	30·9 %
Boston	892	331	37·1	
Lowell	250	4	1·6	
Springfield	185	15	8·1	
Neumann (1902)	206	200	97·0	
Hasslauer (1902)	82	23	28·0	
Stein (1900)	86	7	8·1	
Thomson and Hewlett (1895)	103	0	0	
Baurowicz (1895)	40	0	0	
Children non-contacts.				
Massachusetts Committee (1902)				
Washington	221	1	0·4	25·6 %
Providence	927	140	15·1	
Pugh (1902)	420	234	55·7	
Lack (1899)	100	52	52·0	
Children contacts.				
Massachusetts Committee (1902)				
Minnesota	1154	378	32·7	27·6 %
Brookline	129	15	11·6	
Minnesota Board of Health (1900)	393	70	17·8	
	5238	1477	28·2	

Table B relates only to the distribution of the bacillus in the throats of persons not suffering from clinical diphtheria. As far as possible the observations on adults have been separated from those on children, and those on contacts from those on non-contacts. In both cases it has been impossible to make an entirely satisfactory division, owing to the lack of differentiation in the original tables. According to the statistics of American observers Hofmann's bacillus (Wesbrook's type, D_2) is found only in small numbers in the throats of healthy non-contacts, whether children or adults. Children, however, who have been more or less exposed to diphtheria are more frequently infected. According to the investigations of European observers, Hofmann's bacillus is more commonly present in all classes. They are less numerous in both classes of adults than in children, though in each class more numerous amongst contacts than non-contacts.

Certain investigations on the occurrence of Hofmann's bacillus in the nose are quoted in Table C, mostly by American observers. They indicate that Hofmann's bacillus is more commonly present in the nose than in the throat, and that there is no difference in the degree of infection in contacts and non-contacts, adults and children.

A more careful examination of the former table shows that there is more uniformity amongst the European than the American statistics, but even amongst the former considerable differences are to be found. These differences are probably to some extent due to the different methods employed, and partly to different classes to which the persons belonged. As extreme instances, may be quoted the investigations of Neumann, and Thomson and Hewlett. The former found 98% of the 111 normal noses he examined to be infected, while the latter never found the Hofmann's bacillus in 27 cultures from the vestibulum naris, nor in 76 from the interior of the nose, 101 cultures in all. Very divergent results are also to be found in the American statistics. The collaborators in the "Report on diphtheria bacilli in well persons" by a Committee of the Massachusetts Association of Boards of Health examined the throats of 64 school children and 121 male prisoners at Springfield and found bacilli belonging to type D_2 in 13, to type E_2 in 10, and G_2 in 1, or 24 persons out of 185—(12·9%). At Providence, on the other hand, from the throats of 541 school children, 376 tramps and 10 small-pox patients only 6 bacilli belonging to the type D_2 were found (·6%), and none belonging to types E_2 or G_2—(·6%).

In consequence of the difficulty in interpreting these varying results the writer has added a table (D) showing the results of his findings in the throats of a large number of persons at Cambridge and Colchester. These observations have the advantage of having been made by the same observer under the same conditions. All cultures have been made on a modification of Lorrain Smith's serum, and all have been examined after 12—18 hours' growth and again after a further period of 24 hours' growth. In all cases, wherever possible, single colonies or groups of similar colonies, and not smears, have been microscopically examined. The second examination has frequently resulted in the finding of isolated colonies of the Hofmann's bacillus, which had been missed on the first occasion. The fact that several investigators have confined their examinations to smears made from 18 hour old cultures probably accounts for many low figures, since in crowded cultures containing only one or two very small colonies of the Hofmann's bacillus these are very easily overlooked. Further, in this set of observations pure cultures have been made from a very large number of the colonies showing doubtful organisms and their reaction in glucose broth tested, and the effects of subcutaneous injections into guinea-pigs have been examined in many instances.

As the result of these investigations the writer has come to the conclusion that Hofmann's bacillus is found most commonly in the throats of the children of the poorer classes, especially the scholars in the public schools, the percentage infected being about the same in contacts and non-contacts (51 % and 56 %). The children attending better class schools are less commonly found to harbour this bacillus in their throats (8 % and 15 %). In adults the infection is less than in children, but it is greater amongst the poor (20 %) than amongst the well-to-do (9 %). The Hofmann's bacillus, like the diphtheria bacillus, is probably transferred from mouth to mouth by pencils, sweets, etc., which pass from one child to another, and the habit which children have of placing their fingers and articles, such as pencils, in their mouths accounts for the high infection amongst them with this organism. The absence of such habits amongst older persons may account for the smaller percentage of infection amongst them. In fact a high percentage of infection with Hofmann's bacillus is usually associated with a lack of cleanliness.

Some of the numbers on which these statements are based are very small, for example, only 97 children attending better class schools, and only 66 adults of the more well-to-do class having been examined. The difference in the numbers infected by Hofmann's bacillus in these various classes is, however, sufficiently marked to justify the opinion that more extended investigations along these

TABLE D.

	No. of persons examined	No. in whom Hofmann's bacillus found	Percentage infected with Hofmann's bacillus		Percentage infected with the diphtheria bacillus: Virulent	Not tested	Non-virulent	Remarks.
Adult non-contacts.								
Members of the University	48	2	4·1	9·0	0	—	0	
Workers in the Pathological Laboratory	18	4	22·2		0	—	0	
Patients in Hospital	98	12	12·2	20·6	0	—	1	Mostly belonging to same class as scholars of elementary schools.
Other persons	198	49	24·2		0	—	0	
Adult contacts.								
Colchester	79	18	22·7	19·0	—	29·1	—	
Post-office	52	8	15·3		1·8	—	1·8	
Printing Office	19	2	10·5		0	—	0	
Dress makers	29	7	24·1		3	—	0	
Hospital	40	6	15·0		17·5	—	2·2	
Sanatorium	49	10	20·4		15·5	—	0	
Children non-contacts.								
Cambridge School A	16	10	62·5	55·9	0	—	0	Scholars of public elementary schools.
,, ,, B	21	13	61·9		0	—	0	
,, ,, C	22	10	45·8		0	—	0	
,, ,, D	29	0	0	7·7	0	—	0	Better class schools.
Colchester School XI	10	3	30		0	—	0	
Children contacts.								
Colchester School I	6	4	66·6	51·4	0	—	0	Scholars in the public elementary schools.
,, ,, II	30	20	66·6		0	—	0	
,, ,, III	50	32	64·0		—	16·0	—	
,, ,, IV	49	31	63·3		—	16·0	—	
,, ,, V	149	94	63		—	4·0	—	
,, ,, VI	59	37	62·7		—	8·4	—	
,, ,, VII	16	10	62·5		—	6·2	—	
,, ,, VIII	15	9	60·0		—	33·3	—	
Sturton St School	120	72	60·0		4·1	—	0·8	
Ross ,, ,,	46	28	60·0		2·1	—	0	
Abbey ,, ,,	32	19	59·3		0	—	3·0	
Colchester School IX	37	21	57·0		—	13·5	—	
New St School	42	22	52·3		0	—	2·3	
Catherine St School								
Class 1	47	25 (53·1)	51·1		0	—	0	
,, 2	102	50 (49·0)			1·0	—	0	
,, 3	54	28 (51·8)			14·0	—	1·5	
,, 4	59	27 (45·7)			3·1	—	3·1	
,, 5	41	26 (63·4)			0	—	0	
Park St School	20	10	50·0		0	—	0	
St Matthew's School								
Infants (Spring)	288	105 (36·4)	37·4		5·5	—	1·2	
,, (Autumn)	49	24 (48·9)			14·0	—	0	
Girls	122	43 (35·2)			6·1	—	0·7	
Colchester School XI	9	3	38·3	15·3	—	1·0	—	Better class schools.
Cambridge ,, E	49	6	12·2		—	2·0	—	
	2219	900	40·5					

lines would confirm the value of this classification. Amongst members of the University the infection with Hofmann's bacillus is low (4·1 %). Amongst school children of the same social class the infection in non-contacts was 7·7 % and in contacts 15·3 %. Adult non-contacts, belonging to the class from which the scholars of the public elementary schools are drawn, showed infection in 20·6 % and contacts in 19 %. Amongst school children of this class, who had not been exposed to diphtheria, 55·9 % were infected, whilst amongst those who had been more or less exposed 51·4 % were infected. Many of the scholars included under the head of contacts if exposed at all had only been so to a very slight extent, as may be seen from the absence of diphtheria bacilli amongst them; and only those are called non-contacts who were examined at a time when no diphtheria was present in the town. The uniformity of the results is therefore the more striking. This point is made especially clear in the case of the Catherine Street School, where the percentage of infection with Hofmann's bacillus in all five classes is practically identical, whereas in only three were diphtheria bacilli, virulent or non-virulent, found.

In the throats of 40 % of all persons examined Hofmann's bacilli were found.

Cobbett (IV. 1901, p. 243) had previously made similar observations, remarking that Hofmann's bacillus "was certainly not more frequently found among those who had come into contact with diphtheria than among those who had not. It was less frequently found among the scholars of the 'higher grade' school, where there was much diphtheria, than among the scholars of the ordinary schools, where there was little or none. Among the small number of children of the upper classes examined it was conspicuous by its rarity."

Roux and Yersin (1890) also remarked that these bacilli were less frequently encountered in the better class children.

Cobbett has recently made a very careful examination of the diphtheroid organisms found in cultures from the throats of 90 boys in a reformatory school in which no diphtheria had occurred for some years. Hofmann's bacillus was isolated from the throats of 39 (43·3 %) and was seen in crowded cultures from the throats of some others. All the isolated organs were totally non-pathogenic for guinea-pigs, gave typical cultures on the ordinary media and produced no acid in glucose, galactose, laevulose, maltose, dextrine, lactose, glycerine, saccharose or mannite. This is the most thorough investigation which has yet been made on this subject.

The presence of Hofmann's bacillus has been recorded in many other situations besides the throat and the nose, namely, the eye, the ear, the skin, and the female genitals. This organism, or one very closely resembling it, has also been isolated from pus, the air, and from animal lesions.

The Seasonal Prevalence of Hofmann's Bacillus.

Boycott (1905) has attempted to show that Hofmann's bacillus is more prevalent at certain periods of the year than at others. The materials used were the results of 15,000 examinations made during six years (1899—1904) at the Lister Institute. "The diagnosis is based upon the microscopical appearances found in young (12—20 hours) cultures on serum. The films are made from smears taken over the whole surface of the culture and no attempt is made to pick out individual colonies."

The author states that he has "eliminated from the records those cases in which swabs were taken from persons who were merely 'contacts' of diphtheria infections. The remainder comprise those who were suffering either from true diphtheria or some affection of the throat, bearing a likeness to diphtheria sufficiently close to render bacteriological examination desirable." He concludes that there is a clear difference in the seasonal variations of positive examinations for the two organisms, diphtheria prevailing during September, October and November, while Hofmann's is more frequent from May to August. He discusses the possible errors due to no further search for Hofmann's bacillus being made after the diphtheria bacillus has once been found, and of pure cultures of diphtheria only being obtained when the swabs have been accurately taken from the membrane, and also recognises the fact that Hofmann's bacillus in the latter case may often be present in other areas of the mouth, nose, or pharynx, or, even if present on the swab, be overgrown by the diphtheria bacillus, but thinks that they do not materially affect his results. In spite of these assertions further proof is necessary before these conclusions can be finally accepted, since each of the sources of error mentioned is capable of exerting a considerable influence; and without doubt a more careful method of examination, especially after further growth, would reveal a greater percentage of persons infected with the Hofmann's bacillus. Moreover, if Hofmann's bacillus is a common inhabitant of the healthy mouth and nose, seasonal variations ought to be sought for amongst healthy persons, as well as amongst the class of persons here mentioned, before any definite conclusions can be made.

Summary of Chapter V.

The typical form of Hofmann's bacillus is easily differentiated in young cultures from the diphtheria bacillus by its morphological characters and by its lack of polar bodies when stained by Neisser's method. Even the atypical segmented forms can be readily recognised by a practised observer. In cultures it differs from the diphtheria bacillus mainly by its more luxuriant growth on the common media, and its inability to produce an acid reaction in glucose broth. It is also entirely non-pathogenic to laboratory animals in ordinary doses. The organism is a fairly common inhabitant of the healthy throat and nose, and is more commonly found in children than in adults. Many observers consider that it is more frequently present in the throats and noses of contacts than those of non-contacts, but the writer's experience and that of many other observers is entirely against this view[1].

Note.—Sholley (15. VI. 07) reports that 266 (26·6 %) out of 1000 cultures, obtained from apparently non-exposed children attending the New York hospitals, contained Hofmann's bacilli.

---

[1] Many of the disputed points are discussed in the next chapter.

CHAPTER VI.

THE RELATION OF THE DIPHTHERIA BACILLUS TO HOFMANN'S BACILLUS.

Experimental attempts to convert the diphtheria bacillus into Hofmann's bacillus. Experimental attempts to convert the Hofmann's bacillus into the diphtheria bacillus. Toxoid production by Hofmann's bacillus. Action of the bacilli on various sugars. Agglutination. Haemolysis. Experimental attempts to decrease the virulence of diphtheria bacilli. Experimental attempts to increase the virulence of diphtheria bacilli. Change of virulence by transference from one individual to another. Observations on the change of morphological type by various means. Hofmann's bacilli apparently virulent to man. The virulence towards guinea-pigs of Hofmann's bacilli derived from various sources. Experimental attempts to make Hofmann's bacillus virulent. Change of morphological type in Hofmann's bacillus. Opinions of recent investigators. Summary.

In the last two chapters the principal features of the diphtheria bacillus and Hofmann's pseudo-diphtheria bacillus have been dealt with. In this chapter the various views and arguments as to the relationship existing between them will be considered.

Some observers consider Hofmann's bacillus to be a common innocuous saprophyte, which is in no way related to the diphtheria bacillus. Others appear to think that more than one species of bacillus has the morphological appearance of Hofmann's bacillus and that the diphtheria bacillus is occasionally encountered in this form (see p. 130). Others regard Hofmann's bacillus as a slightly altered and attenuated diphtheria bacillus capable under some conditions of becoming converted into the genuine virulent diphtheria bacillus and giving rise to diphtheria.

Hewlett (1899, p. 203), for example, believing "that Hofmann's pseudo-diphtheria bacillus is a modified diphtheria bacillus," goes so far as to say that a positive diagnosis of diphtheria should be given whenever it is found. The views of the latter school are supported by arguments based on experiments, observations, and statistics.

Some observers have attempted to change the one organism into the other, some have attempted to demonstrate that similar products are produced by the two in culture, and others to show that they react in a similar manner towards agglutinating sera. The opinion is very widely held that diphtheria bacilli recently isolated show great differences in their virulence towards guinea-pigs, and numerous experimenters have sought to increase or decrease the virulence by artificial means. Attempts have been made to prove that one cause of decreased virulence is prolonged stay in the throat. From the results of such experiments it has been argued that the virulent diphtheria bacillus is capable of becoming naturally attenuated and finally converted into the Hofmann's bacillus, and that the Hofmann's bacillus may by a reverse process become changed into a diphtheria bacillus. Arguments in favour of these changes have also been based on statistics apparently showing that Hofmann's bacillus is more commonly found in the throats and noses of those who have recently suffered from diphtheria and those who have been in contact with the disease than in the throats or noses of the normal population.

In this chapter these experiments and deductions are examined at length in the following order.

A. OBSERVATIONS DIRECTLY BEARING ON THE RELATIONSHIP OF THE DIPHTHERIA BACILLUS TO THE HOFMANN'S BACILLUS.

(1) Experimental attempts to convert the diphtheria bacillus into the Hofmann's bacillus.

(2) Experimental attempts to convert the Hofmann's bacillus into the diphtheria bacillus.

(3) Observations on the production of similar substances (toxoids) in culture by the two organisms.

(4) Observations on the action of the two organisms on media containing various sugars and carbo-hydrates.

(5) Observations on the action of agglutinating and bacteriolytic sera on the two organisms: (*a*) sera of diphtheria patients, (*b*) antitoxic sera, (*c*) sera of animals immunised with cultures, (*d*) bacteriolytic sera, (*e*) haemolysis.

Summary.

B. OBSERVATIONS INDIRECTLY BEARING ON THE RELATIONSHIP BETWEEN DIPHTHERIA AND HOFMANN'S BACILLI.

(1) Experiments on the virulence of diphtheria bacilli (*a*) found in the mouths of healthy persons (contacts), and (*b*) on those observed to persist for long periods in the throats of infected persons.

(2) Experimental attempts to decrease the virulence of diphtheria bacilli (*a*) by prolonged cultivation, (*b*) by heating, (*c*) by drying. (*d*) Spontaneous attenuation.

(3) Experimental attempts to increase the virulence of diphtheria bacilli (*a*) by growth with other organisms, (*b*) by passage through animals, and (*c*) by other means.

(4) Observations on changes in virulence in diphtheria bacilli by transference from one individual to another.

(5) Observations on the virulence of diphtheria bacilli causing lesions in different parts of the same individual.

(6) Observations on the changes in morphological type in diphtheria bacilli (*a*) during prolonged persistence in the throat, (*b*) by transference from one individual to another, (*c*) by growth on artificial media, (*d*) by passage through animals.

(7) Observations on the pathogenicity of Hofmann's bacillus. (*a*) Observations on Hofmann's bacilli apparently pathogenic to man. (*b*) The virulence towards guinea-pigs of Hofmann's bacilli recently isolated from (1) healthy persons, (2) persons suffering from diphtheria, (3) convalescents from diphtheria, (4) healthy persons infected with diphtheria bacilli, (5) healthy members of infected families not themselves infected with diphtheria bacilli, (6) persons suffering from throat lesions more or less resembling diphtheria.

(8) Attempts to make Hofmann's bacilli virulent for guinea-pigs (*a*) by passage through animals, (*b*) by growth with other organisms.

(9) Observations on the changes in morphological type in Hofmann's bacilli (*a*) after passage through animals, and (*b*) in culture.

(10) Evidence bearing on the relationship of Hofmann's and diphtheria bacilli derived from preventive measures.

Opinions of recent investigators.

Summary.

A. OBSERVATIONS DIRECTLY BEARING ON THE RELATIONSHIP OF THE DIPHTHERIA BACILLUS TO THE HOFMANN'S BACILLUS.

(1) *Experimental Attempts to convert the Diphtheria Bacillus into the Hofmann's Bacillus.*

Hewlett and Knight (1897, p. 22) attempted to convert diphtheria bacilli into Hofmann's bacilli by heating cultures. They selected a large, segmented, polar staining, acid-forming, virulent diphtheria bacillus, of which a broth culture in a dose of ·25 c.c. was capable of killing a guinea-pig in 24 hours.

Four broth cultures were prepared, and after 48 hours growth' were kept for 4, 6½, 17 and 24 hours respectively at 45° C. The last culture was killed by the heating, but in the first two the result was inappreciable. "The growth in the first subcultures obtained from the broth heated for 17 hours was very poor, showing that most of the organisms had been killed; and the nature of those that remained alive had been completely changed. In later subcultures the bacilli were short, and had lost their polar staining and segmentation." These characters were retained for nine generations (i.e. until the paper was written). Neutral litmus agar was turned blue, and anaerobic cultures in hydrogen gave no trace of growth. Doses of broth cultures up to 5 c.c. proved innocuous to guinea-pigs. The authors conclude their description with the following statement. "We have attempted to obtain the same transformation with other virulent diphtheria bacilli, but not so successfully, although in two instances, after heating, pseudo-forms were obtained; the change from Klebs-Loeffler to pseudo was not nearly so complete. The amount of heating required seems to be a very delicate factor: too little leaves the bacilli comparatively unaltered, a little more kills them completely."

On the other hand, Williams (1902) kept two cultures for several months at 40° C. transplanting them every week, and kept six cultures for several months at 43—45° C., alternating every week to a temperature of 35° C. When growing at 40—43° C. the bacilli were found to be smaller, but returned to their original condition when grown at 35° C.

Park (1900) states that the diphtheria bacillus "may grow at a temperature as high as 41° C. and retain its virulence for months, and Roux and Yersin (1890) found that no morphological alteration of a strain could be affected by growth at 39·5° C.

(2) *Experimental Attempts to convert the Hofmann's Bacillus into the Diphtheria Bacillus.*

Hewlett and Knight (1897) in their experiments made use of typical Hofmann's bacilli, which turned neutral litmus agar blue and produced no effect on guinea-pigs. One experiment is described at great length. The bacillus was obtained from a nurse, who had been nursing a case of diphtheria, and was isolated by passing through agar plates. It was cultivated on serum at 37° C. for 24—48 hours, through 18 generations. During this time its morphology does not appear to have remained constant, for sometimes it appeared to be a typical Hofmann's, at others a short diphtheria, bacillus, and occasionally a mixture of the two types was seen. The 18th generation was a typical Hofmann, producing an alkaline reaction in sugar media, and 5 c.c. of broth culture had no effect on a guinea-pig.

The 19th and 20th generations were each incubated on serum for seven days at 37° C., and from the last a broth culture was made and incubated for six days. Of this 5 c.c. inoculated into a 250 grm. guinea-pig (No. I) caused death in 36 hours with marked oedema. The bacilli were recovered from this animal and broth cultures made from them and incubated for 48 hours killed a 316 grm. guinea-pig (No. II) in 40 hours with marked oedema. The bacilli recovered from the latter killed large guinea-pigs (Nos. III and IV) of 536 and 478 grms. in 36 to 40 hours in doses of 4 c.c. and 3 c.c. The bacilli recovered from these four guinea-pigs were typical diphtheria bacilli in morphology, turned litmus media a bright red, and grew anaerobically in hydrogen. They were capable of killing half-grown guinea-pigs in small doses varying from between 1 and ·25 c.c. The authors discuss, but reject, the possibility of having started with a mixed culture, and state that "in other cases, which showed only typical pseudo-forms, both morphologically, culturally, and in non-virulence, similar treatment, viz. by incubating on serum for a week for a variable number of generations, has resulted in broth cultures a week old, inoculated in 5 c.c. doses, killing the guinea-pigs in 7 to 10 days, or a little longer. This result is so constant, and has been repeated so many times, that we are convinced that it is not merely accidental, especially as guinea-pigs inoculated with similar doses from unincubated cultures usually remain perfectly well." A consideration of the various points raised in their paper leads them "to the conclusion that the so-called pseudo-diphtheria bacillus is frequently a modified and non-virulent diphtheria bacillus."

Trumpp (1896) states that he converted a non-virulent bacillus, which he calls a pseudo-diphtheria bacillus, into one capable of killing guinea-pigs with all the symptoms of true diphtheria by successive passages of cultures mixed with non-fatal doses of diphtheria toxin through guinea-pigs. This organism originally produced acid and was therefore probably a non-virulent diphtheria bacillus.

Levin (1901) said that he converted by cultivation a non-virulent pseudo-diphtheria bacillus into a true virulent diphtheria bacillus, which showed polar bodies. The toxin produced by this organism could be neutralised by antitoxin.

In 1898 Richmond and Salter briefly stated that by repeated passages through certain birds they had been able to convert Hofmann's into diphtheria bacilli, but gave no details. These were supplied by Salter (1899) in the following year.

Nineteen strains of pseudo-diphtheria (Hofmann's) bacilli were examined. All were found to be capable of killing small birds of the finch and bunting families, of which the goldfinch, chaffinch, siskin and sparrow were the least resistant. Sixteen species of birds were experimented on, with the following results:

Goldfinch (*C. elegans*). All 19 pseudo-diphtheria bacilli pathogenic.
Chaffinch (*F. coelebs*). ,, ,, ,, ,,
Canary (*S. canarius*). 18 out of 19 pathogenic.
Siskin (*C. spinus*). 3 pseudo-diphtheria bacilli tested, all pathogenic.
Hedge sparrow (*A. modularis*). 8 pseudo-diphtheria bacilli tested, 6 pathogenic.
Robin (*E. rubecula*). 8 pseudo-diphtheria bacilli tested, 6 pathogenic.
House sparrow (*P. domesticus*). 14 pseudo-diphtheria bacilli tested, 8 pathogenic.
Greenfinch (*C. chloris*). 13 pseudo-diphtheria bacilli tested, 2 pathogenic.
Yellow Hammer (*E. citrinella*). 13 pseudo-diphtheria bacilli tested, 1 pathogenic.
Brambling Finch (*F. montifringilla*). 3 pseudo-diphtheria bacilli tested, 1 pathogenic.
Linnet (*L. cannabina*). 11 pseudo-diphtheria bacilli tested, 1 pathogenic.
Blackbird (*T. merula*). Totally insusceptible.
Thrush (*T. musicus*). ,, ,,
Hawfinch (*C. vulgaris*). ,, ,,
Starling (*S. vulgaris*). ,, ,,
Pigeon (*C. livia*). ,, ,,

It was found that as a result of two passages through a chaffinch any given stock was rendered capable of causing every linnet inoculated to succumb, whilst after two more passages through the latter bird, the thrush and the blackbird were no longer proof against the organism. Similarly, three passages through the goldfinch elevated the virulence of one strain to such an extent that it became capable of killing the blackbird in 48 hours. Finally, it was ascertained that repeated passages through almost any susceptible bird rendered the pseudo-diphtheria bacillus pathogenic to the guinea-pig.

Details of one experiment, in which a non-virulent Hofmann's bacillus was converted into a diphtheria bacillus virulent for guinea-pigs, are given as follows. The organism was: (1) Short and plump, uniform in staining, and without polar granules. (2) Its colonies on serum were pearly white. (3) In neutral broth it produced an alkaline reaction. (4) 10 c.c. of a 48 hour broth culture, in which were emulsified two whole serum cultures, failed to kill a 230 grm. guinea-pig. (5) A filtered 7-day alkaline broth culture in a dose of 20 c.c. was innocuous to a guinea-pig.

Of this organism half a serum culture, suspended in 1 c.c. of a 48 hour broth culture, was inoculated into the left pectoral muscle of a goldfinch. On the second day the bird became quiet and died in 96 hours. Cultures were made from the site of inoculation three hours after death. There was ecchymosis and pallor of the muscle, together with definite turgidity and swelling. Bacilli slightly longer than those inoculated were found in pure culture. Nothing else abnormal was discovered in the body. This culture he labelled A. 1 c.c. of a broth subculture of A was inoculated into a second goldfinch. It died in 48 hours. The organisms found at the autopsy had become longer (culture B). A loopful of B suspended in 1 c.c. of broth was inoculated into a third goldfinch. Death occurred on the third day. At the autopsy the whole of the pectoral muscle on the side of the inoculation was yellowish white and the fibres were very friable and obviously degenerated. The culture obtained was called C. A small loopful of C was injected into a fourth goldfinch. Death took place within 48 hours. The bacilli recovered (D) assumed the typical short diphtheria form with polar bodies.

5 c.c. of a 48 hour culture of D, together with one serum culture, were injected into a guinea-pig of 250 grms. The animal suffered a large local reaction, followed by necrosis, and was ill for several weeks, but ultimately recovered. A tiny loopful of D was injected into a fifth goldfinch. It died in 36 hours. From the local lesion perfect specimens of the diphtheria bacillus were obtained. 5 c.c. of a broth culture, together with a serum culture, killed a 260 grm. guinea-pig on the fourth day with the typical lesions of experimental diphtheria. The pathological effects could be neutralised by antitoxin. Two tubes of alkaline broth were placed side by side, one from the original culture, the other from the exalted culture. The first became alkaline and the

second acid. This experiment was repeated with three other strains. He concludes that "There are to be met with diphtheritic organisms of every grade of virulence. The weakest, known as Hofmann's or the pseudo-diphtheria bacillus, and representing the most attenuated form of the diphtheria bacillus, is capable of killing only certain highly susceptible birds of the finch tribe. Organisms of a slightly higher degree of virulence can kill other and more resistant small birds of the bunting family. Yet others, still more active, can cause larger birds (*Merulidae*) to succumb, while the most virulent of all can kill certain of the rodents, such as the guinea-pig."

The pathogenic action of Hofmann's bacillus on small birds has not been investigated by many observers. Simonin and Benoit (10. I. 1898) state that it is capable of killing a small exotic bird, called "Calfat," and Hewlett (1904) has attempted to enhance the virulence of Hofmann's bacilli by passing them through small birds, but no positive results were obtained. He says that when recently isolated it is virulent for the chaffinch, but by continued cultivation on agar it loses its virulence even for this bird. On the other hand, Williams (1902, p. 107) investigated the subject and found that even in large doses four strains of Hofmann's bacillus and two of the non-virulent diphtheria bacillus were perfectly innocuous to goldfinches.

(3) *Observations on the Production of Similar Substances (Toxoids) in culture by the two Organisms.*

Salter (1899) found that the filtrate of 7—10 days old broth cultures of Hofmann's bacillus although without effect on guinea-pigs in doses of 20—30 c.c., yet in doses of 1—1·5 c.c. was capable of killing chaffinches and goldfinches[1]. Control birds treated with corresponding quantities of sterile broth remained unaffected. He therefore considered that we might legitimately speak of a toxin of the Hofmann's bacillus. He further found that the filtrate, although harmless to guinea-pigs, had in a marked degree the power of neutralising or fixing antitoxic sera. The experiments on which this opinion was based are given below in detail.

A series of seven guinea-pigs were injected with varying quantities of a diphtheria toxin and 1 unit of antitoxin, with the following results:—

---

[1] Salter (1899) states that if a subcutaneous injection of a Hofmann's bacillus be given to a bird a large local gelatinous effusion occurs. Suprarenal reddening is never seen in birds even when inoculated with diphtheria bacilli.

	Weight	Dose	Result
Guinea-pig 1	250 grms.	·35 c.c. of toxin and 1 anti-unit	Died 3 days
,, 2	255 ,,	·34 ,, ,, ,, ,,	,, 3 ,,
,, 3	252 ,,	·33 ,, ,, ,, ,,	,, 4 ,,
,, 4	255 ,,	·32 ,, ,, ,, ,,	Recovered
,, 5	258 ,,	·31 ,, ,, ,, ,,	,,
,, 6	250 ,,	·30 ,, ,, ,, ,,	,,
,, 7	245 ,,	·29 ,, ,, ,, ,,	,,

A second series were treated with similar solutions, containing in addition, however, varying amounts of a filtered broth culture of Hofmann's pseudo-diphtheria bacillus.

	Weight	Dose	Result
G.-pig 8	260 grms.	·32 c.c. of toxin and 1 anti-unit and 1 c.c. of pseudo-diphtheria filtrate	Died 3 days
,, 9	250 ,,	·32 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	,, 3 ,,
,, 10	245 ,,	·32 ,, ,, ,, ,, ·25 ,, ,, ,, ,,	,, 4 ,,
,, 11	255 ,,	·31 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	,, 4 ,,
,, 12	248 ,,	·30 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	,, 5 ,,
,, 13	252 ,,	·29 ,, ,, ,, ,, ·25 ,, ,, ,, ,,	,, 8 ,,
,, 14	250 ,,	·29 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	Recovered
,, 15	255 ,,	·28 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	Died 6 days
,, 16	240 ,,	·26 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	Recovered

He states that the toxin antitoxin mixture was always prepared first and allowed to stand for a few minutes, before the pseudo-diphtheria filtrate was added. The filtrate must therefore have possessed the capacity of breaking up the neutralised combination, liberating the toxin, and taking its place; that is to say, it was a Protoxoid.

Lesieur (1901) alleges that certain strains of "pseudo-diphtheria" bacilli, although non-virulent for guinea-pigs in ordinary doses, can nevertheless cause fatal paralyses similar to those due to diphtheria toxin, provided that large doses of recently isolated cultures be used. He evidently appears to consider that the paralysing substance is a very similar body, if it is not indeed identical, with the toxone in diphtheria toxin. These experiments have, however, not been confirmed by more recent observers.

Cobbett (1903, *Journ. of State Med.* p. 609) and Hewlett (1904) have been unable to find any toxoids in cultures of Hofmann's bacillus. The most thorough and convincing observations are those of Petrie (1905), who, working with 11 races of Hofmann's bacillus, mostly derived from diphtheria patients or contacts, which showed no polar granules by Neisser's method, produced no acid in glucose broth, and were non-virulent to guinea-pigs in doses of 5 c.c., made several experiments to ascertain whether any toxoid was produced.

One race (No. 1) was grown in a large volume of alkaline broth in the usual manner for the production of diphtheria toxin, and a second culture of the same race was grown in Martin's pig-stomach bouillon at 36° C., and after 10 days' growth the cultures were filtered through Pasteur-Chamberland filters. A mixed filtrate from the other 10 races was also prepared. Tables are given in his paper showing the results of the 33 experiments similar to those made by Salter, carried out with a view to ascertaining whether toxoids were present in the filtrates. A quantity of filtrate was first added to a unit of antitoxin and the mixture then placed in the incubator for 15 minutes before the addition of the toxin. In this way any toxoid which might be present had an opportunity of combining with the antitoxin, thus preventing a corresponding amount of toxin from entering into combination. A further series of 11 experiments were made in which the dose of toxin was such that toxone effect became manifest in 3 or 4 weeks. He comes to the conclusion that "Taken as a whole these experiments may be interpreted as indicating that no toxoids were present in the filtrates of the pseudo-diphtheria (Hofmann's) bacilli examined." Taking the view that if toxoid is really present as a product of metabolism of Hofmann's bacillus the immunisation of a suitable animal with these products ought to lead to the production of an antitoxin in the animal's serum, he made the following experiments. Three horses were repeatedly injected with large quantities of the two filtrates mentioned. Before and after the experiments their blood was tested for antitoxin and none was found. Consequently the filtrates employed were not capable of producing an antitoxin to diphtheria toxin. As the result of his experiments Petrie concludes: (1) that no substances capable of neutralising diphtheria antitoxin are present in filtrates of Hofmann's pseudo-diphtheria bacilli, and (2) that the results of the immunisation of horses with large quantities of the filtrates make it apparent that they do not contain substances capable of stimulating the production of an antitoxin to diphtheria toxin.

Glücksmann (1897), Bergey (1898) and others had previously ascertained that the immunisation of animals with cultures of Hofmann's bacillus did not confer any protection against diphtheria bacilli injected subsequently, and Spronck (1896) had found that the local reaction produced in guinea-pigs by the subcutaneous injection of large doses of pseudo-diphtheria bacilli was not influenced by the injection of antitoxin. One or two instances are, however, quoted, in which a very slight degree of protection seems to have been produced against diphtheria bacilli by immunisation with Hofmann's bacillus.

(4) *Observations on the Action of the two Organisms on Media containing Various Sugars and Carbohydrates.*

Experiments have already been quoted (p. 159) which show that diphtheria bacilli, whether virulent or non-virulent, derived from clinical cases, convalescents or contacts, invariably produce acid in the presence of glucose, galactose and laevulose, generally with maltose, glycerine, dextrine and lactose, and occasionally with saccharose, but never with mannite, when these substances are contained in Hiss's serum medium or in broth free from muscle sugar. These experiments give no indication that the power of reacting on these substances is gradually lost during a prolonged stay in the throat[1].

Hofmann's bacillus, on the other hand, does not produce acid in the presence of any of these substances under similar conditions. If the contention that Hofmann's bacilli are merely attenuated diphtheria bacilli be true, it would seem probable that some of these organisms, when isolated from the throats of clinical cases or convalescents, would still be capable of producing acid with glucose. The experiments of many observers prove that this is not the case. During the last five years the writer has systematically isolated all the morphologically atypical forms of this organism, and large numbers of typical forms, and tested their reaction on glucose broth, but has never found a race capable of producing acid. Recently (Graham-Smith, 1906) experiments were also made with 20 races on media containing the substances mentioned above. Three of these races were derived from clinical cases of diphtheria, four from convalescents, four from contacts also infected with diphtheria bacilli, three from persons suffering from sore throat, and the rest from healthy throats and noses. None of these races showed any power of forming acid from any of these substances. These experiments, therefore, do not lend any support to the view that Hofmann's bacilli are merely attenuated diphtheria bacilli.

---

[1] Some writers say that the acid producing power of the diphtheria bacillus is uncertain and of no value in differentiation, but most of them seem to refer to its action on ordinary broth without the addition of glucose. Under such conditions whether acid is produced or not depends on the quantity of muscle sugar present.

In connection with these experiments a statement by Theobald Smith (1896) is of interest. He says: "An extended experience in testing the acid producing function of bacteria leads me to state that if, as has been claimed, the pseudo-diphtheria bacilli do not act on dextrose, there exists a most profound difference between them and true diphtheria bacilli, sufficient to separate them once and for all."

(5) *Observations on the Action of Agglutinating and Bacteriolytic Sera on Diphtheria and Hofmann's Bacilli.*

Numerous observations have been made with the view of ascertaining whether diphtheria bacilli can be differentiated from other diphtheria-like organisms by their reaction to the sera of patients suffering from diphtheria, antitoxic sera, or the sera of animals treated with cultures. No satisfactory results have been yet obtained with the first two sera. One of the difficulties in this method of investigation is the tendency of diphtheria bacilli to adhere in smaller or larger masses, and the impossibility of making completely homogeneous emulsions.

(*a*) *The action of the sera of diphtheria patients.*

Bruno (1898) investigated the agglutinating action of the sera of 44 diphtheria patients on young cultures of the diphtheria bacillus, both by the hanging drop and sedimentation methods. Two of these sera had no agglutinating action, 23 agglutinated in dilutions varying from between 1 : 10 and 1 : 80, 18 acted at about 1 : 100, and one at 1 : 400. He found that the serum of healthy individuals never had an agglutinating value above 1 : 30, but that after the injection of antitoxin it rose to double this value in some cases.

(*b*) *The action of antitoxic sera.*

Landsteiner (1897), Nicolle (1898), and Blandi (1903) failed to discover any agglutinating power in antitoxic serum. Nicolas (1896–98 and 1900), Fraenkel (1896), and Lesieur (1901) all found some agglutinating action, but it was inconsistent and variable and of no service in differentiating the organisms.

(*c*) *The action of the sera of animals immunised with cultures.*

Lubowski (1900) obtained an agglutinating serum by injecting a goat with non-virulent diphtheria bacilli. The bacilli were emulsified in ·85% salt solution by vigorous shaking together with glass, and the suspension thus obtained was thoroughly mixed with 10% glycerine solution. Of this 1 c.c. was placed in a small test-tube and mixed with 1 c.c. of a suitable dilution of serum. After thorough mixing the contents were poured into a small Petri dish and examined under a low power. If the emulsion was satisfactory, it was put into an incubator and examined from time to time. The mean time of reaction was 1—2 hours. By this method 23 races of typical virulent diphtheria bacilli were examined and two races of non-virulent diphtheria bacilli. All these were agglutinated in dilutions of 1 : 80, and in some cases up to 1 : 160. Neither cocci nor Hofmann's bacilli (three races) were agglutinated. Normal serum was found in some cases to agglutinate up to 1 : 40.

Lesieur (1901) studied the action of the sera of horses, immunised by cultures of diphtheria bacilli, on 40 races of diphtheria, and 30 of pseudo-diphtheria bacilli, and found that while certain varieties of diphtheria bacilli were agglutinated others were not. The pseudo-diphtheria bacillus behaved in the same manner. He considers that the fact constitutes a new presumption in favour of the identity of certain species of pseudo-diphtheria bacilli with the true diphtheria bacilli, but as 50 (29 virulent and 21 non-virulent) of his 70 races were not agglutinated the only allowable deduction seems to be that the agglutination reaction has no value as a means of distinguishing between them.

Schwoner (1902) immunised a horse first with dead and then with living cultures of diphtheria bacilli, and obtained a highly powerful agglutinating serum. For the purpose of control he also experimented with normal horse and antitoxic sera. The latter two agglutinated between 1 : 10 and 1 : 40. He found that all the 50 races of diphtheria bacilli with which he worked were agglutinated by his serum up to 1 : 500 and some even up to 1 : 10,000. It, however, only agglutinated pseudo-diphtheria bacilli in the same strength as normal horse or antitoxic sera. Other organisms, such as *B. coli*, *B. typhosus*, and *V. cholerae* were also agglutinated at this dilution. He also immunised a goat with a pseudo-diphtheria bacillus. Its serum agglutinated that race in 1 : 10,000, but not four other strains of pseudo-diphtheria bacilli or any of his diphtheria bacilli. As the result of his observations he comes to the following conclusions: (1) That an agglutinating serum of high value can be obtained from diphtheria bacilli. (2) That a difference can be obtained between true and pseudo-diphtheria bacilli. (3) That a pseudo-diphtheria agglutinating serum can also be obtained; and (4) That the pseudo-diphtheria bacillus is not one true race.

One of his tables shows 45 races of diphtheria bacilli agglutinated in dilutions of 1 : 1000 to 1 : 4000 in every case, and another table shows 14 races agglutinated in dilutions of 1 : 5000 and 1 : 10,000.

In 1903 Schwoner made some further experiments with 12 strains of pseudo-diphtheria bacilli. He describes 11 races of his pseudo-diphtheria bacilli, some of which are apparently Hofmann's and others non-virulent diphtheria bacilli. He divides them into two groups:—

(*a*) Culturally and morphologically Hofmann's bacilli which produce an alkaline reaction in broth, a good growth on agar, and can be easily differentiated from diphtheria bacilli.

(*b*) Morphologically like diphtheria bacilli producing a poor growth on agar and a slightly alkaline, or even acid, reaction in broth.

He made a polyvalent serum in a goat from four races of Hofmann's bacilli which agglutinated these four strains and three others in dilutions of 1 : 1000. The other races were not agglutinated. Three monovalent sera, made by injecting a goat and two rabbits, only acted well on the races from which they were made.

Gordon (1902) made agglutinating sera in guinea-pigs by the injection of killed cultures. He found that a diphtheria serum agglutinating in a dilution of 1 : 100 did not agglutinate Hofmann's bacillus, and that similarly a Hofmann agglutinating serum did not act on diphtheria bacilli. One diphtheria agglutinating serum reacted on four out of six freshly isolated virulent diphtheria bacilli and one non-virulent diphtheria bacillus, but had no action on two diphtheria-like organisms, or on Hofmann's bacilli or *B. coryzae segmentosus*.

Blandi (1903) also obtained a diphtheria agglutinating serum by the intraperitoneal injection of heated cultures.

Schick and Ersettig (1903) worked with 50 races of diphtheria bacilli. Of these 12 were tested for virulence and all killed within 72 hours. They obtained an agglutinating serum from a horse, which agglutinated all 50 races in dilutions of 1 : 2000, but had no effect on various other bacilli.

Martin (1903) obtained sera reacting at 1 : 200 by intravenous or intraperitoneal injections of bacilli killed by heating to 100° C. He found that the most convenient method of carrying out the agglutinating experiments was with bacilli heated in fluid to 100° C. for one hour and emulsified with water or normal saline solution. The coarser particles were allowed to sediment and the upper layers used.

Lipstein (1903) obtained agglutinating sera from rabbits by the injection of virulent bacilli together with antitoxic serum. He found that the sera so obtained acted in high dilution only on the race used to immunise the animal, and not on other races of diphtheria bacilli. When two races were used for immunisation the resulting serum agglutinated them both in equal dilutions, but not other races. He explains these results by supposing that all races have certain receptors in common, but that in addition each race has receptors peculiar to itself.

Bacteriolytic sera.

Lambotte (1902) injected guinea-pigs first with emulsions of diphtheria bacilli killed by heating to 60° C., and later with living cultures at eight day intervals. The serum was then found to contain a specific bacteriolysin for diphtheria bacilli. The serum of guinea-pigs treated with Hofmann's bacillus contained a specific bacteriolysin for that organism. He found that the serum for one organism acted to some extent on the other, and consequently thinks that they are connected.

Haemolysis.

Lubenau (1901) observed that cultures (six out of seven) of diphtheria bacilli were capable of producing haemolysis. The maximum haemolytic power was shown in broth cultures at periods varying between 1 and 13 days.

Schwoner (1904) made more extended observations on 70 cultures. He found that the haemolytic power was best shown on rabbits' corpuscles, and as a result of his experiments concluded that:—

(1) It is chiefly strains of diphtheria bacilli from clinically severe septic cases which give haemolysis; (2) the power is at its maximum in 48 hour broth cultures; (3) the property is lost by long cultivation; (4) filtrates of cultures show no haemolysis; (5) the pseudo-diphtheria bacillus does not possess any lysin.

Summary of Section A.

In the preceding pages it has been shown that Hewlett and Knight lay claim to having on one occasion converted by means of heating a virulent diphtheria bacillus into a Hofmann's bacillus. These authors, however, were apparently unable to repeat the process, and Williams was unable to confirm their results, and no other observers have recorded changes in cultures kept at high temperatures. Hewlett and Knight, by prolonged cultivation, and Salter, by passage through small birds, consider that they have converted Hofmann's bacilli into diphtheria bacilli. No subsequent observers, however, appear to have been able to repeat successfully the former experiments, and, although Hewlett states that he has found the Hofmann's bacillus pathogenic to chaffinches, Williams denies that it has any action on the goldfinch, the most susceptible of all birds according to Salter.

At present therefore too much importance must not be attached to these experiments unless they can be successfully repeated.

In regard to the production of culture products by the Hofmann's bacillus of a nature similar to those of the diphtheria bacillus, Salter considered that the Hofmann's bacillus formed a protoxoid. No subsequent observers have found any trace of this substance, and the exhaustive experiments of Petrie appear to demonstrate conclusively that no such substance is produced. Other observations show that no immunity against the diphtheria bacillus can be produced by means of the Hofmann's bacillus. These experiments therefore, so far as they go, point to the non-identity of the two organisms.

The observations on the power of the two organisms to produce acids from certain sugars and carbohydrates prove that in this respect they are totally distinct. While the diphtheria bacillus is capable of acting on several of them, Hofmann's bacillus is incapable of producing acid in the presence of any of them.

The observations of Lubowski, Schwoner, Gordon, Schick and Ersettig on agglutinating sera all point to a specific difference between the two bacilli, and so do the haemolytic experiments of Schwoner. Although Lesieur thought that his observations were in favour of the identity of the two organisms, his experiments are of very doubtful value. Lambotte's bacteriolytic experiments seem to show that the two organisms are connected, but not specifically identical.

The observations relating to the formation of toxoids, acid production, and the action of agglutinating sera, with few exceptions, tend to show that diphtheria and Hofmann's bacilli belong to different species, only related to each other in being members of the same group.

B. OBSERVATIONS INDIRECTLY BEARING ON THE RELATIONSHIP BETWEEN DIPHTHERIA AND HOFMANN'S BACILLI.

(1) *Experiments on the Virulence of Diphtheria Bacilli found*

(*a*) *In the mouths of healthy contacts.*

It has already been shown that the great majority of observers consider that diphtheria bacilli recently isolated from clinical cases display considerable differences in their virulence towards guinea-pigs. Further, it has been constantly asserted that the diphtheria bacilli, which remain after the clinical symptoms are over, gradually lose their virulence, and may at length after a prolonged stay in the throat become completely non-virulent to laboratory animals. In fact it has been generally considered that diphtheria bacilli of all grades of virulence may be met with in the throats of convalescents and other persons. By many it has been further supposed that the representatives of the originally virulent diphtheria bacilli after becoming changed into non-virulent diphtheria bacilli undergo further changes and become Hofmann's bacilli.

In view of these statements it becomes important to consider how far the reputed change in virulence is supported by experimental evidence. Certain reasons have already been given for thinking that intermediate stages of virulence are probably of much less common occurrence than has generally been supposed, and that lack of uniformity in methods may account for many of the statements which have been made. The bacilli obtained from patients showing all the symptoms of clinical diphtheria are usually found to possess a high degree of virulence, and it is unnecessary to quote the numerous experiments which have been made. Mention must, however, be made to the fact that attenuated and non-virulent bacilli have on rare occasions been isolated from such cases.

In the great majority of cases the bacilli found in the throats of persons who have come into contact with diphtheria patients, but show no signs of the disease, are also fully virulent, though some are non-virulent. The virulent bacilli of the patients do not therefore seem to suffer a diminution in virulence by transference to immune individuals. The results of the observations of some of those workers, who have tested the virulence of the bacilli obtained from recent contacts in a considerable number of cases, are quoted below.

Park and Beebe (1894) examined 48 healthy children in 14 infected families and found diphtheria bacilli in over 50%. Of six cultures tested all were virulent. Also five out of six infected contacts discovered in a foundling hospital showed virulent bacilli.

Aaser (1895) in an outbreak of diphtheria in a cavalry regiment examined 89 well persons. Seventeen of these harboured diphtheria bacilli, and all were fully virulent. He also examined the contacts in two scarlet fever wards in which cases of diphtheria were present. All the diphtheria bacilli which he isolated and tested were virulent.

Bolton (1896) amongst 214 persons who had been more or less exposed to diphtheria found the virulent bacillus in 41·5 %.

Müller (1897) examined the children in a general ward into which cases of diphtheria had been admitted. Twelve children with diphtheria bacilli were discovered. Six of these were virulent and six attenuated or non-virulent.

Kober (1899) found that 15 out of 128 contacts showed the presence of diphtheria bacilli, all of which were virulent.

Cobbett (1901) tested for virulence nine pure cultures obtained from 19 contacts. Of these six were fully virulent and three entirely non-virulent.

Graham-Smith (1904) during an outbreak at Cambridge isolated and tested for virulence the diphtheria bacilli found in 56 well persons who had come into more or less close contact with cases of diphtheria. Of these 38 harboured fully virulent, and 18 totally non-virulent, diphtheria bacilli[1].

Pennington (1907) tested the bacilli obtained from 18 undoubted contacts, using 24 hour serum cultures for inoculation. One strain was fully virulent, 10 more or less attenuated, and seven totally non-virulent.

Table showing the virulence of diphtheria bacilli isolated from recently infected contacts:

Observer	No. of diphtheria bacilli tested for virulence	Fully virulent	Totally non-virulent or causing a slight infiltration
Park and Beebe	12	11	1
Aaser	17	17	0
Meade Bolton	88	88	0
Müller	12	6	6
Kober	15	15	0
Cobbett	9	6	3
Graham-Smith	56	38	18
Pennington	18	1	7
	227	182 (80·1 %)	35 (15·3 %)

---

[1] For details of these experiments see Chapter XII.

These records clearly show that the majority of well persons harbouring diphtheria bacilli, which have been derived by contact from clinical cases, retain in their throats fully virulent organisms, and that the transference of a virulent diphtheria bacillus from a diseased to an immune person does not tend to weaken its virulence.

There is also some evidence to show that non-virulent diphtheria bacilli when transferred from one person to another do not become virulent, or give rise to the disease. In the Cambridge outbreak just mentioned the writer (Graham-Smith 1904) found in three households 5, 4, and 2 persons respectively harbouring non-virulent diphtheria bacilli without the clinical signs of diphtheria. They were only examined by accident, as two of these persons had slight sore throats. Further 13 other persons with non-virulent diphtheria bacilli remained at their homes and constantly mixed with other persons. No clinical cases of diphtheria are known to have arisen from such persons. Cobbett (1901) had a similar experience, and Park and Beebe (1895) state that typical non-virulent diphtheria bacilli are probably incapable of causing diphtheria, for the 24 cases in which they were found by them never developed any lesions, nor were they the origin of any case of diphtheria, so far as could be ascertained.

(b) *Observations on the virulence of diphtheria bacilli persisting for long periods in the throats of infected persons.*

In regard to the alleged general decrease in virulence by prolonged stay in the throats of infected persons certain experiments show that a decrease has probably taken place, but many other experiments very conclusively prove that the organisms may persist in the throat or the nose for very prolonged periods without showing any decrease in virulence. A number of these observations are given in the following pages.

Tobieson (1892) tested for virulence 19 strains of diphtheria bacilli found up to 31 days after the disappearance of the membrane. Of these 16 strains killed guinea-pigs between 24 and 50 hours. Two caused tumours and the animals died later. One produced early necrosis, paralysis after five weeks, and death in seven weeks. Dowson (1893) observed fully virulent diphtheria bacilli in the noses of two children during five months; and Belfanti (1894) found a boy harbouring fully virulent diphtheria bacilli seven months after the attack. Ritter (1894) observed fully virulent diphtheria bacilli in one-third of 324 diphtheria cases after the disappearance of the membrane. In four cases they were present five weeks after the disappearance of the membrane. Gladin (1895) found virulent diphtheria bacilli present in two cases for 33 days. In another case non-virulent diphtheria bacilli were found on the 45th day. Schäfer (1895) discovered virulent bacilli in the throat of a boy 7½ months after an attack of tonsillitis. Martha (1895) found in the throat of a patient nine weeks after the attack bacilli which caused oedema but did not kill, and came to the conclusion that their virulence had been decreased by treatment.

Sevestre and Méry (1895) observed two cases in which virulent diphtheria bacilli were present 49 and 38 days after the disappearance of the membrane, and Symes (1895) in a case of rhinorrhoea, following scarlet fever, found virulent diphtheria bacilli for seven weeks. Washbourn and Hopwood (1895) found virulent diphtheria bacilli in the throat of a nurse for six weeks. Williams (1896) observed a case in which diphtheria bacilli persisted for 157 days. Up to the last they were fully virulent.

Hewlett (1896) isolated from a boy, 22 weeks after an attack of diphtheria, bacilli which in doses of ·5 c.c. and ·25 c.c. killed 500 grm. guinea-pigs in 36 hours, and Müller (1897) observed fully virulent diphtheria bacilli in the mouth of a contact for 2½ months. Stone (1897) tested 18 strains of diphtheria bacilli isolated from cultures obtained from persons 3 weeks after the disappearance of all clinical symptoms. The bacilli were grown for 48–120 hours in sugar free broth, of which 1 c.c. was injected into 800 grm. guinea-pigs. Fourteen strains were virulent killing between 24 hours and 7 days, the majority within 3 days.

Smith and Walker (1897) made careful experiments on the toxin producing power of 11 races of diphtheria bacilli obtained from the throat at periods varying between 15 and 62 days from the beginning of the disease. The results of these observations are given in the following table:

Interval in days from the earliest symptoms	Minimal fatal dose of toxin
17	·12 c.c.
15	·08
27	·08
42	·08
57	·08
62	·08
16	·07
22	·07
22	·06
26	·05
62	·05

They finally say "we observe that, so far as toxin production is concerned, the length of time the bacilli have sojourned in the throat has no tendency to reduce it below the average."

Russel (1899) in two patients found virulent diphtheria bacilli 59 days and 4½ months after the disappearance of the membrane.

Cobbett (1901) went further than the majority of the observers who have been quoted, and isolated and examined for virulence the bacilli from a number of persons on several occasions until their disappearance. Bacilli were isolated and found virulent in one person on the 2nd, 23rd, and 30th days, and in another on the 1st, 30th, 36th, and 66th days. In two contacts the bacilli were found to be virulent in one on the 15th, 36th, and 58th days and in the other on the 1st, 18th, 28th, 60th, 69th, and 82nd days. The bacilli from three other contacts who harboured non-virulent bacilli were also tested on more than one occasion, and were always found to be non-virulent. The bacilli from one of these were examined on the 1st and 31st days, from another on the 24th and 34th days, and from the third on the 1st, 21st, 24th, 34th, 63rd, 65th, 67th, 84th, 88th, and 93rd days.

Prip (1901) examined the bacilli found after the disappearance of the membrane in eight persons up to their final disappearance. In these cases full virulence was maintained up to the time of the disappearance of the bacilli, in one case up to 335 days. In two other cases, however, the bacilli showed a decrease in virulence. In one case the bacillus isolated on the 142nd day was virulent, but non-virulent on the 530th day, and in the second case although virulent bacilli were obtained on the 13th day, those isolated on the 293rd day were non-virulent.

Williams (1902) found that the bacilli from convalescent cases, isolated two and sometimes three times to test their virulence, retained their pathogenic properties until their final disappearance. Pure cultures were always isolated from the first serum cultures as well from the tubes containing the last diphtheria bacilli, each day's tubes containing 24 hours' growth being kept in the ice box until the next cultures were examined.

Pugh (1902, p. 308) found virulent diphtheria bacilli in the throat of a nurse for nine weeks after she had been nursing a case of diphtheria. This nurse had never suffered any impairment of health, and continued to harbour the virulent bacilli in spite of a month's vigorous local treatment, followed by some weeks at the sea-side.

Wesbrook (1905) found virulent diphtheria bacilli present in three cases on the last occasion on which they were isolated and tested, namely on the 46th, 72nd, and 80th days.

Pennington (1907) tested the bacilli obtained from 25 convalescents. "In several instances the organisms were isolated at different times during convalescence and their virulence tested for a diminution in activity. In only one case was such a loss of virulence noted."

In the following table the longest periods of persistence of *fully virulent diphtheria bacilli* recorded by various observers is given:

Prip	335 days	Pugh	63 days
Schäfer	280 ,,	Smith and Walker	62 ,,
Belfanti	215 ,,	Symes	49 ,,
Williams	157 ,,	Sevestre and Méry	49 ,,
Hewlett	154 ,,	Washbourn and Hopwood	42 ,,
Dowson	150 ,,	Ritter	35 ,,
Russel	137 ,,	Gladin	33 ,,
Cobbett	82 ,,	Tobiesson	31 ,,
Wesbrook	80 ,,	Stone	21 ,,
Müller	75 ,,		

The observations which have just been quoted conclusively prove that diphtheria bacilli are capable of retaining their virulence during very prolonged persistence in the throats of infected persons. In a few instances they appeared to have lost their virulence to some extent, but in the great majority they have retained their full virulence up to the time of their final disappearance.

(2) *Experimental Attempts to decrease the Virulence of Diphtheria Bacilli.*

With the majority of pathogenic bacteria it is possible by various means to increase or decrease their pathogenic powers, and working on these lines many observers have sought to influence the virulence of the diphtheria bacillus. Although these experiments when successful only prove that alterations of virulence can be brought about by artificial means, many seem to have regarded the results as lending support to the view that the virulent diphtheria bacillus is related through the non-virulent diphtheria bacillus to Hofmann's bacillus.

(*a*) *By prolonged cultivation on artificial media.*

Many observers have noticed that even after very prolonged cultivation on artificial media the diphtheria bacillus does not tend to show any appreciable loss in pathogenic properties, though some have noticed a diminution in certain instances. Williams (1902, p. 105), for example, grew one culture for six years on artificial media and found that there was an apparent slight loss of virulence. Since "its virulence on broth averaged one two-hundredths cubic centimeters" this apparent loss of virulence after very prolonged cultivation is of little importance. Three other strains of bacilli grown on artificial media for one year all retained their original virulence. Two other cultures grown for some time on hard-boiled egg showed distinct morphological changes, the bacilli becoming exceeding short and looking like diplococci, but when transferred to other media they immediately regained all their original characteristics.

Peters (1896) found that long diphtheria bacilli did not show any decrease in virulence after prolonged cultivation, but says that the short diphtheria bacillus readily loses its virulence on culture. Bardach (1895) found that a culture lost some of its virulence after two years' artificial cultivation.

(*b*) *By heating.*

It has already been shown that Hewlett and Knight on one occasion succeeded in completely changing the morphology and virulence of a diphtheria bacillus by heating cultures, but were unsuccessful in later attempts. On the other hand Williams (1902) was unable to alter the virulence by heating, even to 45° C., and Park (1900) found that virulence was retained for months at 41° C. Roux and Yersin (1890) observed that by cultivation on broth at 39·5° C. for a month virulent diphtheria bacilli could apparently be deprived of their virulence. They found that broth cultures kept at 39·5° C. for 25 days did not kill when injected directly, but subcultures from these flasks killed quickly. Consequently no real attenuation had taken place. On repeating these experiments in a slightly different way attenuation however resulted. Broth cultures were grown at 39·5° C. until obvious development had occurred. The temperature was then raised to 40° C. In one case after 13 days of such treatment a subculture only caused oedema, and in another instance subcultures were non-virulent after 23 days. They consider that loss of virulence only occurs shortly before the death of the organisms.

(*c*) *By drying.*

Roux and Yersin (1890) observed some diminution in virulence in bacilli kept for a very long period of time in dried membranes. In one case numerous colonies of virulent diphtheria bacilli were obtained from a membrane after a month's drying, but after five months only attenuated bacilli were found. Bacilli from another membrane kept under the same conditions showed no sensible diminution in virulence after three months.

Golowkoff (1895) thought that the virulence of bacilli kept on cloth was diminished, but Abel (1895) on the other hand found no decrease in virulence in bacilli dried on silk threads after 86 days.

(*d*) *Spontaneous decrease in virulence.*

Roux and Yersin (1890) twice observed rapid spontaneous loss of virulence in culture.

(3) *Experimental Attempts to increase the Virulence of Diphtheria Bacilli.*

The observations recorded in this section deal only with attempts to raise the virulence of living diphtheria bacilli for animals when injected in the usual manner, and not with attempts which have been made to produce an increase of the production of toxins in culture media. The latter question is dealt with later (Section V).

(*a*) *By growth with other organisms.*

Several attempts have been recorded to increase the virulence of diphtheria bacilli by cultivating them together with other organisms for more or less prolonged periods. Most of these experiments, of which one noteworthy example is quoted, have yielded negative results. Williams (1902) grew two morphologically typical, but non-virulent, strains of diphtheria bacilli together with virulent streptococci through 90 generations, transplanted every three or four days, but when separated no change in virulence or other characteristics were noted.

(*b*) *By passage through animals.*

Williams (1902) attempted to give virulence to several strains of non-virulent diphtheria bacilli by successive peritoneal inoculations, but in no instance did any increase in virulence occur. She also passed two non-virulent morphologically typical bacilli through goldfinches, but the organisms when recovered from the birds were still non-virulent for guinea-pigs.

Lubowski (1900) and Bergey (1898) could not make non-virulent diphtheria bacilli virulent by repeated passages through guinea-pigs, and Fibiger (1895) was unable to raise the virulence of non-pathogenic diphtheria bacilli by injecting them together with streptococci. Cobbett (1901, p. 497) found that a minute abscess sometimes formed at the site of inoculation after the injection of non-virulent diphtheria bacilli. "From these little abscesses the bacilli in pure culture were several times obtained and tested on guinea-pigs to see if they had gained in virulence. One of them was passed through four animals in succession. In each case the injection of 2c.c. of 48 hours broth culture produced no more effect than at first.[1]"

The writer has recently experimented with five strains of morphologically typical non-virulent diphtheria bacilli. These were injected subcutaneously into white rats, and three were recovered after 2, 2 and 5 days. Attempts to recover the other two strains after 2 and 3 days failed, and in no case could the bacilli be cultivated from the blood and organs. The recovered bacilli were found to be totally non-virulent to guinea-pigs in doses of 2 c.c. of 48 hour broth cultures.

Park (1900) says that "the passage of diphtheria bacilli through the bodies of susceptible animals does not increase their virulence to any considerable extent, this being probably due to the fact that the bacilli multiply but little in the tissues."

Although the attempt to give virulence to entirely non-pathogenic diphtheria bacilli has usually been unsuccessful, many investigators claim to have considerably raised the virulence of bacilli which had either been only feebly virulent when first isolated, or had subsequently become so.

Martin (1898) passed bacilli through the peritoneum of the rabbit in collodion sacs, and was enabled by this treatment to augment the toxigenic power, though the passage did not increase the virulence.

Roux and Yersin (1890), though they were unable to raise the virulence when it was very low, could raise to some extent the virulence of cultures which caused oedema, but did not kill, by passing through animals together with virulent streptococci. These exalted cultures subsequently retained their virulence. Bardach (1896) succeeded in slightly raising the virulence of a culture, which had become attenuated by two years' growth on artificial media, by numerous passages through dogs, and he states that he was able to raise the virulence for rabbits of bacilli already virulent for these animals by repeated passages through them.

---

[1] In this connection Cobbett says that he has frequently seen similar abscesses form in guinea-pigs treated with large doses of virulent bacilli together with antitoxin, and also in an immunised horse treated with living bacilli. In the latter case the bacilli had retained their virulence.

Spronck (1895) found that the virulence of a bacillus which originally killed guinea-pigs in two or three days in a dose of ·2 c.c. could be raised by three passages through these animals to such an extent that a dose of ·1 c.c. killed in 24 hours.

Aronson (1893), Funck (1895) and Martin (1898) all considered that the virulence of diphtheria bacilli which were already virulent could be raised by passages through animals. Ohlmacher (1895, footnote) makes the following observations: "In my own work I have not attempted to recover the bacilli from the seat of inoculation, but believing that the bacilli which gained access to and survived in the visceral organs were the most virulent, I have recovered them from these organs. In this way I was able, for instance, by a single passage through a guinea-pig to make a diphtheria bacillus, which only killed a guinea-pig in 14 days, become so virulent that it killed an animal of equal weight in 30 hours in a much smaller dose." In experiments published later (1902) he states that a short, uniformly staining and slightly virulent bacillus (killing in seven days) after its passage through an animal became long and granular and more virulent.

(c) *Increase of virulence by other means.*

Concetti (1901) states that by anaerobic growth he caused a non-virulent streptothrix-like diphtheria bacillus to become pathogenic and toxic.

Shattock (1898) found that lowly virulent diphtheria bacilli did not acquire virulence when grown in a current of sewer air, even for a period of two months.

(4) *Observations on Changes in Virulence in Diphtheria Bacilli by Transference from one Individual to another.*

The ordinary process of transference of the disease from one individual to another, and the fact that diphtheria bacilli isolated from recent contacts are virulent in 80 % of the specimens tested, prove that in the majority of cases virulent bacilli when transferred retain their virulence. The experimental and natural transference of fibrinous rhinitis (Chapter VIII) also suggests this view. Under natural conditions, owing to the difficulty of tracing the path of infection, the proof of loss or of increase of virulence, if any occurs, in the passage of a bacillus through a series of individuals is not easy to obtain, unless the type is a particularly distinctive one. In one such instance, in which a very remarkable type infected several individuals in succession in a home for destitute children, Williams (1902, p. 101) observed no change in virulence, although many of the children showed no clinical manifestation of the diphtheria.

Gorham (1901, p. 208) asks "is it not possible that a culture isolated from a healthy throat has lost its virulence for guinea-pigs because of its exposure to the fluids of an immune body and that later, when under the proper conditions in the throat or nose of a susceptible human being, may regain its virulence and be capable of producing clinical diphtheria?" On this subject certain observations have been made from time to time (p. 233), which indicate that non-virulent but otherwise typical diphtheria bacilli do not acquire virulence for guinea-pigs, or give rise to diphtheria when transferred from one individual to another.

In some instances the examination of a person with a slight sore throat, who has not been recently exposed, reveals diphtheria bacilli, which turn out to be non-virulent. In these cases cocci or other organisms are usually also abundant, and probably account for the disease.

(5) *Observations on the Virulence of Diphtheria Bacilli causing Lesions in Different Parts of the same Individual.*

Very few observations have been made on whether diphtheria bacilli, almost certainly derived from the same source, producing diphtheritic lesions in different situations in the same individual, are ever of different types, or show different degrees of virulence. Williams (1902, p. 97) records the examination of two such cases: "The first was a throat case, which had also a vaginal discharge. The same variety of virulent diphtheria bacilli were isolated from cultures from each locality. The second case had throat symptoms and a membrane on a finger wound. In this instance, too, the same variety was isolated from both cultures."

The writer has recently found the same type of fully virulent diphtheria bacillus in the throat and in an ulcer of the foot of a patient, who had been exposed to diphtheria.

(6) *Observations on the Changes in Morphological Type in Diphtheria Bacilli.*

(*a*) *During prolonged persistence in the throat.*

Several observers have stated that they consider that their examinations of convalescent patients lend support to the view that the typical diphtheria bacilli, which are alone found during the acute stages of the disease, gradually lose their characteristics after a more or less prolonged stay in the throat and are finally represented by uniformly staining types, of which Hofmann's bacillus is the furthest removed from the virulent diphtheria bacillus. This view, they say, is also supported by the statistics of the occurrence of Hofmann's bacillus in the throat and nose. According to them Hofmann's bacillus is rarely to be found in clinical cases, but is very frequently present in convalescents, and much more common in those who have been exposed to diphtheria than those who have not. Statistics have already been quoted (p. 205) on the occurrence of Hofmann's bacillus in the throats of contacts and non-contacts, which show great discrepancies amongst the observations of various workers. The general conclusion is, however, that this organism is equally distributed amongst exposed and non-exposed persons in the various grades of the community. The frequency of its occurrence in clinical cases has, however, yet to be dealt with.

Many observers state that it is rarely to be met with in clinical cases, but seldom mention whether their view is based on any other evidence than the examination of cultures from the diseased part. They frequently seem to have lost sight of the fact that, if the swabs are accurately taken from the membrane in clinical cases, few organisms but diphtheria bacilli are likely to be met with in the cultures; even if they were originally present in this situation they would probably be overgrown by the rapidly forming and very numerous colonies of the diphtheria bacillus. The fact of their non-appearance under these conditions cannot, therefore, be taken as proof of the absence of Hofmann's bacilli in other parts of the buccal or nasal cavities.

Further, it is seldom stated how thoroughly the cultures have been searched for colonies of Hofmann's bacillus. If few colonies only are present the probability of overlooking them is very great owing to their small size, and to their similarity to those of the diphtheria bacillus. Smears made from the general surface can be of little value, since the individual specimens of Hofmann's bacillus are mixed up among the diphtheria bacilli and may easily be overlooked, if few, or mistaken for young specimens of the diphtheria bacillus. A further period of incubation of the cultures often results in the differentiation of the colonies of Hofmann's and diphtheria bacilli sufficient to make an examination at that period of greater value, but such subsequent examinations seldom seem to have been made.

Once the diphtheria bacillus has been found most observers are satisfied to record its presence, and make a diagnosis.

For these reasons the majority of opinions which have been recorded as to the absence of Hofmann's bacillus in clinical cases are of little value.

Observations dealing with the frequent occurrence of Hofmann's bacilli in the throats and noses of convalescents are numerous, but the deductions founded on these observations cannot be regarded as of much value, unless the observers have previously ascertained by adequate experiments whether the bacilli were present in the throat or nose at the height of the disease, and have made a sufficient number of experiments on normal persons of the same class to determine their frequency amongst such persons.

In very few experiments have satisfactory experiments been made on these points.

Hewlett and Knight (1897) state that Hofmann's bacillus replaces the diphtheria bacillus during convalescence, since "almost always the pseudo appears towards the end, the next examination or so revealing an absence of either diphtheria or pseudo-diphtheria bacilli." In order to illustrate their statement they give a list of 24 cases in which the diphtheria bacillus was apparently replaced by Hofmann's bacillus.

Wesbrook, Wilson and McDaniel (1900) also thought that the typical diphtheria bacilli were replaced by uniformly staining forms till finally the D^2 or Hofmann type became the most common or the only type present.

This statement they illustrate by several examples recording the various types found in the course of routine diagnoses of cultures for the establishment and maintenance of quarantine. Cases 1 and 2 showed the following types[1] according to their classification in a series of cultures taken during a period of about three weeks:—

---

[1] See p. 130, Plate VIII.

Case 1.

Exam. number	Types present
1	C, D, E
2	A, C, D
3	A, C, D
4	A, C, D
5	A, C, D, E
6	A, D, D^1, E, E^2
7	A, B^1, D, D^1, D^2, E
8	D^2, D^1

Case 2.

Exam. number	Types present
1	A, C, D
2	A, C, D, E
3	A, C, D, D^2, E^2
4	A^1, C^1, D^1, D^2, E^2, G^2

The authors remark that "these cultures show (*a*) the presence of large granular types, with absence of other types, during the prevalence of clinical symptoms, and (*b*) the entire replacement of granular types by barred and solid types as convalescence was established and just prior to the entire disappearance of all diphtheria-like organisms." They go on to state that "this seems to be the usual order of variation in clinical cases, as has been noted by other observers and frequently in this laboratory." They give also an example in which exactly the opposite conditions were found, and say in regard to it that "such cases in the experience of this laboratory are infrequent, but that they do occasionally occur is beyond doubt."

No. 3.

Exam. number	Types present	Exam. number	Types present
1	D^2, E^2	4	D, D^2, E^2
2	D^2, E^2, D^1, E^1	5	D, C, A
3	D^2	6	D, C, E, A

They recorded the changes in about 200 cases, and came to the conclusion that the variability of the granular types of bacilli is greater than that of the solid forms.

Gorham (1901) says that in his opinion there seems to be no question that Wesbrook's types, including D^2, are all varieties of the diphtheria bacillus, and considers that during convalescence the typical forms of the diphtheria bacillus become converted into uniformly staining types or Hofmann's bacilli. He asks "Can we not conclude that the granular or barred ($C\,C^1$) are the natural forms of virulent diphtheria bacilli, and that these forms under the influence of body fluids of persons not susceptible to the diphtheria toxin, or who are becoming slowly immune, gradually become non-virulent, and in doing so change to the solid staining types C^2 and D^2?......If this sequence of form is dependent on the interaction of bacillus and patient, then the same changes of form ought to be shown by a series of examinations of patients who have had clinical diphtheria and have recovered."

To illustrate this change he gives a record of the examinations of 10 cases, all of which at some period show the presence of uniformly staining types.

Williams (1902), on the other hand, made a very careful study of the types found in cultures during successive examinations of 10 cases of diphtheria. "In studying these cases we see that different types appear irregularly throughout the course of the disease, but no sequence can be observed. When pure cultures were isolated from a tube containing these different types it was found that exactly the same variety of bacillus, and only this variety, was obtained as from the earlier culture, showing that the new forms are due, at least in part, to the influence of other bacteria, and it is only when we study a pure culture that we can obtain a true idea of the variety to which the individual bacillus belongs."

"In this series of cases no pseudo forms appeared in the throats throughout the course of the disease that were not there at the beginning." "In these cases the typical diphtheria bacilli from the original nose cultures showed slight differences from those from the throats of the same cases; pure cultures, however, showed that the same variety was present in both throat and nose."

"Some of the pure cultures had such persistent characters that their recognition was particularly easy, and there could have been no question, even to the most superficial observer, in regard to their maintaining the same type throughout the course of the disease."

In conclusion she remarks: "In regard to the appearance of solidly staining pseudo-forms towards the end of diphtheria I would say further that in an examination of hundreds of control smears made in routine work for pronouncing diphtheria cases at the Willard Parker Hospital free from diphtheria bacilli, only occasionally were pseudo-forms observed, and in all of the cases where the typical bacilli persisted for some time and were isolated two and sometimes three times to test their virulence, the same variety continued to be present unmixed with new atypical forms."

As the result of a series of experiments on diphtheria and diphtheria-like bacilli, extending over seven years, this author comes to the conclusion "that not only are there distinct species in this group, but that each species has distinct subspecies or varieties with characteristics which continue to persist under different conditions; these varieties as well as subspecies remain separate, and, when grown under similar conditions, the species show no tendency to become converted into one another, while the varieties gradually change, approaching a common form. The term variety is applied to a pure culture as a whole and not to individual bacilli, and by it is meant that certain pure cultures possess some persistent morphological and cultural characteristics so different from those of other pure cultures, that the culture has a distinct individuality."

The writer has also frequently noticed in many consecutive examinations for release from quarantine that the same type of bacillus is to be found till its final disappearance. In fact in certain instances, in which the organisms had some marked peculiarity, a glance at a preparation was sufficient to tell from what culture it was obtained. Some of these bacilli were very long, others had an unusual number of stained segments, others large numbers of polar bodies, and a few belonged to the "sheath" variety. A few have been met with persisting for very long periods, which were peculiar in that they produced no visible colonies before 36 hours' incubation. He has also frequently come across cases in which, throughout a long series of examinations, Hofmann's bacilli were never found. In one instance, for example (No. 26, Table, Chapter XII), 50 consecutive examinations were made in 122 days from the throat of a child and Hofmann's bacillus was never encountered. During the whole of this period a morphologically typical diphtheria bacillus was present in the throat. In another case (No. 71) virulent diphtheria bacilli were present for 65 days and ultimately disappeared, but Hofmann's bacillus was never found in 24 examinations. Several other examples may be seen in the table mentioned.

Cobbett (IV. 1901, p. 251) in his records relating to the serial examinations of 11 cases of notified diphtheria, found Hofmann's bacillus at some period in four (36%), and in similar examinations of eight infected contacts found it five times. He also came to the conclusion that Hofmann's bacillus does not replace the diphtheria bacillus.

The statement that Hofmann's bacilli are frequently encountered during convalescence is probably due to the fact that at this period the culture tubes are no longer crowded by innumerable colonies of the diphtheria bacillus, and colonies of other organisms begin to make their appearance. As convalescence advances colonies of the diphtheria bacillus become rarer, and a prolonged search has often to be made. In the course of such examinations colonies of Hofmann's bacillus are met with, and the impression is produced that this organism has replaced the diphtheria bacillus.

According to the statements of several observers the age of the culture, slight differences in the composition of the medium, the presence of contaminating bacteria, and other factors may influence to some extent the type of the bacillus found in the original cultures from the throat or nose. In making any observations on the changes of type some of these factors, especially the possible influence of other bacteria, ought to be eliminated by the preparation of pure cultures.

The important observations of Williams on the constancy of the types in pure cultures prepared from successive cultures from convalescents, from persons infected in two situations, and from persons infected from the same source have already been quoted.

Cobbett (x. 1901, p. 495) also made a similar statement. Some Hofmann's bacilli "as they first appear on the original culture, are far more than others difficult to distinguish on morphological grounds from true diphtheria bacilli, and in not a few instances I have been in doubt until pure cultures have been isolated. However much they may have resembled the diphtheria bacillus at the start, they come in subcultures closely to resemble what I regard as the typical form."

Cobbett's observations on this question the writer has corroborated by a very large number of experiments. Colonies have been very frequently met with, which, together with the typical Hofmann forms, contain varying numbers of segmented bacilli, and occasionally some which consist entirely of segmented bacilli. Such segmented bacilli have a more or less characteristic appearance, and can be differentiated from true diphtheria bacilli by their morphological and staining characters (see p. 200). Pure cultures have been made from such colonies on very many occasions, and also from a very large number of colonies containing a few doubtful bacilli. Almost without exception the young pure cultures have consisted entirely of bacilli of the typical Hofmann form, and in very many cases, in which they were further tested, they produced no acid in glucose broth and no effect on guinea-pigs, even in large doses. According to Wesbrook's method the cultures which have just been mentioned would probably have been recorded as containing type D^2, as well as types D^1 and C^1, and probably in a few cases A^1.

These variations may be to some extent due to the influence of other bacteria or mucus, and are also certainly due sometimes to the composition of the medium, for they have been especially frequently observed in one or two batches made from human serum. The value of the preparation of pure cultures in such cases for diagnostic purposes cannot be over-estimated, and it is also of great importance in considering the alleged changes of morphological type amongst diphtheria bacilli.

Comparative observations on the occurrence of Hofmann's bacillus in the throats of clinical cases and healthy infected contacts at some period during examinations for release from quarantine, in the throats of contacts not infected with diphtheria bacilli, and of non-exposed persons belonging to the same class of the community, have seldom been made during an outbreak. Observations of this character are, however, more likely to give to the observer a true idea of the distribution of Hofmann's bacillus and its connection or lack of connection with the diphtheria bacillus than any number of repeated examinations of cultures from convalescents alone.

Cobbett (1901) records during one outbreak the results of consecutive examinations of 18 persons, who either suffered from clinical diphtheria, or harboured morphologically typical diphtheria bacilli, and amongst these nine (50%) at some period showed Hofmann's bacilli. Amongst 650 other persons examined, some of whom had not been exposed, Hofmann's bacilli were found in 157 (24·1%). Although the precise figures are not given the author states that "it was certainly not found more frequently among those who had come into contact with diphtheria than those who had not. It was less frequently found among the scholars of the 'higher grade' school, where there was much diphtheria, than among the scholars of the ordinary schools, where there was little or none. Among the small number of children of the upper class examined it was conspicuous by its rarity. When it is remembered that the infected persons were examined on the average six times each and some only showed a few colonies at one examination, it seems probable that had the healthy persons been examined as often the incidence of this organism amongst them might have been as high as among the infected."

In another outbreak (x. 1901), investigated several months later, he confirmed the opinions he had previously formed.

Graham-Smith (1904, p. 297), in an extensive outbreak at Cambridge, found that the proportion of persons infected with Hofmann's bacillus was nearly the same among convalescents recovering from diphtheria (48%), contacts infected with diphtheria bacilli (51·9%), non-infected contacts (43·7%), and non-contacts (54·2%).

Nearly all these persons were children belonging to the same social class. Amongst adults, whether infected with diphtheria bacilli or not, the percentage of infection with Hofmann's bacilli was much smaller.

During a previous outbreak at Colchester (Graham-Smith, 1903) 112 persons, notified as suffering from diphtheria, were examined on the average six times each for release from isolation. Some of these were first examined after the disappearance of the diphtheria bacillus. Hofmann's bacillus was at some time present in cultures from 62 (55·3 %) of them. Amongst all the persons examined in the town, whether exposed or not, it was present in 31 %, but 63 % of the scholars of the public elementary schools, amongst whom most of the notified cases of diphtheria occurred, harboured this organism. On further analysing these records, however, it was found that the percentage infected with Hofmann's bacillus was the same amongst scholars attending schools in which diphtheria was common as amongst those attending schools in which few or no cases occurred.

The records of this outbreak agree therefore with those of the one just quoted in showing that Hofmann's bacillus is as common amongst normal school children attending the public elementary schools as amongst others who are recovering from diphtheria or have been exposed to it.

(*b*) *By transference from one individual to another.*

Many instances are to be found recorded in the literature of patients infected from the same source harbouring bacilli similar on culture. The writer has frequently noticed the same type of diphtheria bacillus in all members of an infected family, and in one instance of a small outbreak in a village found that every infected person, whether showing clinical signs or not, harboured very long bacilli with many segments. Williams (1902) records some observations in an outbreak in a home for destitute children bearing on this point. Two deaths had occurred in this institution, but the type of bacillus had not been recorded. Later an outbreak of sore throats began, and cultures from all cases were found to contain the same variety of virulent bacillus as the first case. The variety was peculiar in that many coccus-like forms appeared when the organisms were grown on agar. It was also peculiar in producing very delicate colonies on agar, and a very scanty growth in broth. Observations such as these show that changes in morphological type do not occur when diphtheria bacilli are transferred from one individual to another.

(*c*) *By growth on artificial media.*

It has already been shown that both diphtheria and Hofmann's bacilli exhibit differences in morphology more or less great when grown on different media. When, however, the organisms are again sown on serum the original type appears. The question here to be discussed is, whether by means of growth on artificial media the characters of any given race of bacilli can be so altered that when grown on serum at 37° C. its morphological appearances are markedly affected. It seems to be universally accepted that by more or less prolonged growth slight changes in morphology are brought about. The experiments of Hewlett and Knight would lead us to suppose that by prolonged cultivation alone the morphological type and even biological character of diphtheria-like bacilli can be profoundly altered. The observations of several other observers have caused them to believe that the morphological types of diphtheria bacilli are unstable and can readily change in culture. Wesbrook, Wilson and McDaniel (1900), for example, state that "attempts to classify types of bacilli on the appearance of microscopical fields of presumably pure cultures had to be abandoned," and Ohlmacher (1902) believes that most of the common characteristics of the diphtheria bacillus are unstable.

The former observers give several examples, some of which are quoted below, of the change of form in pure cultures. "When the observations here recorded were begun, the stocks had been isolated by a repetition of the process of colony picking and subsequent streaking out from three to fifteen times. Each subculture having been made in the same way, it is apparent that the stocks at the time of the last observation had been subjected to this purification process from twenty to forty or more times."

Pure stock No. 2 (clinical case).

No. of culture		Morphological types in 16—18 hour cultures
1.	Original throat culture	C, D
2.	Pure culture*	B, C^1, C, D^2
3.	,, ,,	D, E, B, D^2
4.	,, ,,	B, C^1
5.	,, ,,	A, D, E
6.	,, ,,	B, A, C, C^1
7.	,, ,,	B, C, A, C^1
8.	,, ,,	A, C, E

* Two years after isolation.

Pure stock No. 5 (non-clinical case).

No. of culture		Morphological types in 16—18 hour cultures
1.	Original throat culture	C, D, E^2
2.	Pure culture†	B, C, C^1
3.	,, ,,	C, D, E, E^2
4.	,, ,,	A, B, C, D, E, E^2
5.	,, ,,	A, C
6.	,, ,,	A, C, D
7.	,, ,,	C^1, B, A
8.	,, ,,	C, D, C^1, A

† Eight months after isolation.

In stock No. 2 the general tendency is to the predominance of the long forms throughout. The culture remained virulent for guinea-pigs throughout its whole history. In stock No. 5, which was obtained from a child who had not recently been exposed to diphtheria, the large granular types predominate, but it was never possible to kill a guinea-pig even with large doses.

Pure stock No. 6 (clinical case).

No. of culture		Morphological types in 16—18 hour cultures
1.	Original throat culture	D^2
2.	Pure culture	D^2
3.	,, ,,	E^2, D^2
4.	,, ,,	G^2
5.	,, ,,	E^2, G^2
6.	,, ,,	E^2, D^2
7.	,, ,,	E^2, G^2
8.	,, ,,	D^2

This stock, although there were never any other than solid types present, was apparently a true diphtheria bacillus, and killed guinea-pigs not protected with antitoxin.

Hadley (1907) considers that his "observations permit of little doubt that a single morphological variety of the diphtheria organism is decidedly modifiable; and that not only may the granular types of the organism be resolved into the solid staining forms, but the opposite may also be true."

In opposition to these views are those put forward by certain other observers, who believe that certain types are very stable and that pure cultures of them possess some persistent morphological character. Williams (1902) kept pure cultures of a large number of diphtheria bacilli on agar at 36° C. transplanted every two to four weeks, and 10 cultures of Hofmann's bacilli for the same time in broth and on serum. At the end of that time when transplanted upon other culture media the individual characteristics of the cultures were unchanged. Also several different varieties of diphtheria-like bacilli were kept on media for six months to a year, and all retained the characteristics of the original cultures. Certain diphtheria bacilli with peculiar characters already described (p. 249), obtained from numerous individuals in an outbreak at a school for destitute children, were observed through numerous generations for eight months, but showed no changes in morphology. Williams concludes from her observations that pure cultures of diphtheria bacilli continue to show the same characters through many culture generations and that there are many distinct varieties of diphtheria-like bacilli, all of which in serial pure cultures retain the characters of the original culture. The writer has also often noticed that pure cultures of diphtheria bacilli kept growing on serum for numerous generations continue to show the same general morphological characters as the original cultures. He has also found that diphtheria bacilli grown for 10 days at 37° C. in media containing different sugars and carbohydrates, when transplanted on to serum, showed the same morphological peculiarities as the original cultures.

(*d*) *By passage through animals.*

By some observers certain changes in morphological type have been noted in bacilli recovered from the tissues of animals. Hadley (1907) found that a virulent D^2 type, when recovered from the tissues of a guinea-pig, became barred. On the other hand three strains, two showing CC^1 types and one C^1C^2 types, after recovery only showed D^2 type. The most remarkable examples have been quoted by Ohlmacher (1902). He experimented with three organisms, and concluded that by a short sojourn in an immune animal a diphtheria may be converted into a Hofmann-like bacillus, and that the reverse may be brought about by passing the organism through a susceptible animal. He states that a long granular diphtheria bacillus after recovery from the subcutaneous tissue of a rat became short and uniformly staining, that a uniformly staining, but pathogenic, bacillus after recovery from the spleen of a guinea-pig became granular, and a short uniformly staining Hofmann-like bacillus after its passage through a guinea-pig became long and granular. The statements of Ohlmacher cannot be accepted as universally true, as Williams (1902), experimenting on four varieties of diphtheria bacilli, found no changes in morphological type after they had been in the body of an immune host (white rat) for 48 hours. The same observer also found no change in morphological type in non-virulent diphtheria bacilli after successive intraperitoneal inoculations through guinea-pigs. The writer has recently experimented with 10 strains of diphtheria bacilli, five virulent and five non-virulent. These have been recovered after two to five days' sojourn in the subcutaneous tissues of white rats, but no differences in morphology were noted. In two cases small abscesses had formed, and these also contained bacilli of the same type as those injected.

(7) *Observations on the Pathogenicity of Hofmann's Bacillus.*

If, as has frequently been asserted, Hofmann's bacillus is merely an attenuated and slightly altered diphtheria bacillus, capable under certain conditions of regaining its virulence and giving rise to diphtheria, it might be expected that in the process of attenuation before complete loss of virulence had been attained, or on the other hand during the process of regaining pathogenic power before sufficient virulence had been acquired to produce typical diphtheritic lesions, the organism would be capable of setting up some disease in man, and giving rise to lesions in guinea-pigs, animals peculiarly susceptible to diphtheritic poison.

Observations dealing with these points, so far as they bear on the relationship of diphtheria and Hofmann's bacilli, are considered under the following headings: (*a*) Observations on Hofmann's bacilli apparently pathogenic to man. (*b*) The virulence towards guinea-pigs of Hofmann's bacilli recently isolated from (1) healthy persons, (2) persons suffering from diphtheria, (3) convalescents from diphtheria, (4) healthy persons infected with diphtheria bacilli, (5) healthy members of infected families not themselves infected with diphtheria bacilli, (6) persons suffering from throat lesions more or less resembling diphtheria.

(*a*) *Observations on Hofmann's bacilli apparently virulent to man.*

Many observers are of the opinion that Hofmann's bacillus is capable of giving rise to disease in man. The opinions, however, vary as to the real nature of such conditions. While some consider that the disease is in reality mild diphtheria, others think that it is a specific affection produced by the Hofmann's bacillus. Others again, while admitting that this organism is not infrequently encountered in diseased conditions, consider that it is not in any way related to them.

Hewlett and Knight (1897) quote several histories to show that Hofmann's bacillus is associated with mild anginas, which are free from complications, end in recovery, and are not followed by sequelae. Richmond and Salter (1898) found it to be the only organism present in an outbreak of post-scarlatinal diphtheria in a hospital ward, and Priestly (quoted by Caijer, 1904) records an outbreak of throat-illness in a school at Lambeth characterised by tonsillar exudation, in which the Hofmann's bacillus and no other was present in 38 out of 43 of those attacked. These are only examples of records of a similar nature, many of which are to be found in the literature. It will be pointed out later (Chapter XI), however, that diseases simulating diphtheria occur, the causal agents of which do not grow on the ordinary media under the usual conditions. The presence of Hofmann's bacillus in culture does not therefore necessarily prove, unless such other organisms have been excluded, that it is the causal agent. Further, even if organisms resembling Hofmann's bacillus in morphology are shown to be the only ones present in large numbers, their cultural and other characters must be worked out before their identity can be established.

Wesbrook, Wilson, and McDaniel (1900), for instance, found in an outbreak of diphtheria at Owatonna that the type of diphtheria bacillus most frequently present closely resembled the Hofmann's bacillus. It could, however, be differentiated from the latter by the fact that it formed acid in broth, and a strong toxin, whose effects could be neutralised by antitoxin. They also seem to have encountered the same type of diphtheria bacillus on other occasions.

Ruediger (1903) and later Hamilton (1904) have also recorded the presence in severe throat affections of organisms, apparently belonging to different species, which, although they somewhat resembled Hofmann's bacillus in morphology, differed in their cultural and pathogenic properties (Chapter X).

The evidence supporting the view that Hofmann's bacillus is capable of producing disease in man is almost entirely based on the presence in cultures from the throat of organisms morphologically identical with this bacillus. Most observers have only examined young serum cultures and have not excluded the possibility of the lesions being due to other organisms, which either grow slowly on serum or refuse to grow on it, or to other organisms, such as *B. fusiformis*, which cannot be cultivated aerobically, and only with difficulty in anaerobic cultures. Bearing in mind the extremely common occurrence of Hofmann's bacillus in the throats and noses of healthy persons, and the fact that this organism is non-pathogenic to guinea-pigs, animals peculiarly susceptible to the products of the diphtheria bacillus, observations of a more convincing nature, which exclude the presence of all other known pathogenic organisms, are needed before its capacity for producing disease in man can be regarded as proved.

(*b*) *The virulence towards guinea-pigs of Hofmann's bacilli recently isolated from*

(1) *Healthy persons.*

Gorham (1901, p. 209) says that "type D_2, frequently called the atypical form of *B. diphtheriae*, is probably to be included in the 'pseudo-diphtheria' or 'Hofmann' group of many writers. It has been shown to be frequently pathogenic to guinea-pigs, and, when so, protection is afforded by the use of commercial antitoxin."

The Massachusetts Committee (1902, p. 18) in commenting on this question make the following statement: "Both these observers (Gorham and Wesbrook) several times isolated solid forms in pure culture from healthy persons, and proved them virulent for guinea-pigs. Such a condition, however, must be very rare, as no other members of the Committee have ever found these forms present alone in clinical diphtheria; and when isolated by them from healthy persons, they have always proved to be non-virulent."

The experiences of almost all other observers are in agreement with this statement.

Park (1898), although he has apparently met with two cultures of the "pseudo type" which were virulent, states (1900, p. 351) that Hofmann's bacilli "never produce diphtheria toxin," and "that no facts have come to light which indicate that bacilli, which do not produce diphtheria toxin in animals, ever produce it in man."

Amongst those who have made the most numerous experiments are Cobbett and Neumann. Cobbett (4. x. 1901) isolated and tested 86 strains, and found them all to be non-virulent in doses of 2 c.c. of 48 hour broth cultures, and Neumann (1902) inoculated into guinea-pigs in doses of 2 c.c. of 48 hour broth culture 78 strains obtained from normal and diseased noses without any effect.

During the course of the last five years the writer has isolated and tested on guinea-pigs a large number of bacilli obtained from healthy persons, which though they differed slightly from typical Hofmann's bacilli in the original cultures, resembled them in sub-cultures in every morphological and cultural character, including the production of an alkaline reaction in glucose broth. All without exception proved non-virulent to guinea-pigs.

(2) *Persons suffering from diphtheria.*

Cobbett (IV. 1901) isolated in at least two cases both Hofmann's bacilli and diphtheria bacilli from original serum cultures obtained from patients suffering from clinical diphtheria, and proved the former organisms to be non-virulent. In a few instances the writer (Graham-Smith, 1904, p. 291) has also isolated Hofmann's bacilli from the original serum cultures taken from clinical cases of diphtheria at the height of the disease[1]. In all these cases the bacilli have produced an alkaline reaction in glucose broth, and doses of 2 c.c. of well-grown 48 hour broth cultures have been without effect on guinea-pigs. On the other hand the diphtheria bacilli isolated from the same cultures have been fully virulent. Petrie (1905) also isolated three races from patients suffering from diphtheria and found them to be typical in all respects, and totally innocuous to guinea-pigs.

---

[1] Other details concerning some of these cases are given in Chapter XII. (Table. Nos. 11, 12, 24, 39, 54, 88.)

(3) *Convalescents from diphtheria.*

Cobbett (IV. 1904) isolated Hofmann's bacilli from the throats of three convalescents when diphtheria bacilli were still present, and found them to be typical in all respects and totally without virulence for guinea-pigs, and Williams (1902) made similar observations. On several occasions (six) the writer has also isolated Hofmann's bacilli from the throats of convalescents at an early stage, and has never found them to produce any greater effects than a transient oedema in guinea-pigs.

(4) *Healthy persons infected with diphtheria bacilli (infected contacts).*

As in the case of patients suffering from diphtheria and convalescents, the writer has isolated and tested a number of strains of Hofmann's bacilli from healthy persons infected with virulent or non-virulent diphtheria bacilli, and has always found them to be non-pathogenic to guinea-pigs. Cobbett (IV. 1901, p. 249) also isolated Hofmann's bacilli from the throats of infected contacts and proved them to be non-virulent, and similar observations have been made by a number of other workers.

(5) *Healthy members of infected families not themselves infected with diphtheria bacilli.*

Hofmann's bacilli, isolated from healthy non-infected members of families in which diphtheria had recently occurred, have been shown by several observers to be totally non-virulent for guinea-pigs.

(6) *Persons suffering from throat lesions more or less resembling diphtheria.*

Cobbett (IV. 1901) reports two instances in which Hofmann's bacillus was the only diphtheroid organism isolated from cultures taken from persons clinically suffering from diphtheria. In neither case did the organisms show any indications of virulence towards guinea-pigs, although 2 c.c. of three, four, and seven day old broth cultures were injected. Petrie (1905) isolated two strains from suspected cases of diphtheria and found them to be typical Hofmann's bacilli in all respects, and innocuous to guinea-pigs.

For some time past the writer has isolated and tested on animals all examples of Hofmann's bacillus obtained from persons suffering from lesions resembling diphtheria sufficiently closely to make a bacteriological examination desirable, and in whom no diphtheria bacilli have been found. Twenty-six such strains have been inoculated into guinea-pigs without producing any results. Smears made direct from the swab were examined in a number of these cases, some of which showed fusiform bacilli and spirilla, and some large numbers of cocci, etc. It is possible that in some of these cases a multiplication of the Hofmann's bacilli present in the normal condition takes place secondary to the inflammation set up by the other organisms, since very large numbers of colonies are sometimes found. In other cases only a few colonies of this organism develop, not more numerous than are frequently to be obtained from the normal throat. No experiments appear to have been made with the view of ascertaining whether Hofmann's bacilli, frequently present in the normal throat, multiply during inflammatory processes due to other organisms. Nevertheless, it is a matter of some interest in forming an opinion in regard to the causal connection between Hofmann's bacilli and some of the conditions in which they are found. Whether or not such a multiplication is proved to occur, it must be borne in mind that innumerable colonies of Hofmann's bacilli can frequently be obtained from the healthy nose.

Summary of Section 7.

The observations which have been quoted in this section show that diphtheria bacilli, which in other respects are typical of the species, may in very rare instances morphologically resemble Hofmann's bacilli, and that other pathogenic species more or less resembling Hofmann's bacilli, but easily distinguishable by their cultural characters, may produce lesions in man. These facts are in no way opposed to the general conclusion from the other facts recorded, that morphologically typical Hofmann's bacilli which are culturally characteristic producing no acid in glucose broth, show (according to almost all observers) no virulence to guinea-pigs even when inoculated in large doses.

If Hofmann's bacillus is merely an attenuated form of the diphtheria bacillus, incapable of producing lesions in guinea-pigs when cultivated from the throats and noses of healthy persons, specimens isolated from persons suffering from diphtheria, convalescents, and infected contacts should exhibit some signs of virulence towards guinea-pigs when injected in comparatively large doses. Again, if, as Gorham appears to think possible, the fluids of an immune person are capable of changing a virulent diphtheria bacillus into a Hofmann's bacillus shortly after infection, some of the latter, when discovered in the mouths of healthy members of infected families, and therefore according to this view probably recently converted diphtheria bacilli, ought to show traces of virulence. Under none of these conditions, however, is any trace of virulence shown. Finally this bacillus, when discovered as the only diphtheroid organism in mild, or even severe, throat or nose lesions is without virulence for guinea-pigs. Most of such diseased conditions are probably due to other bacteria, and there is little evidence in support of the view that Hofmann's bacillus is even capable of producing non-diphtheritic inflammatory lesions in man. In fact the weight of the evidence, so far as guinea-pig experiments are of value, points to the conclusion that under all conditions Hofmann's bacillus is a harmless saprophyte.

(8) *Attempts to make Hofmann's Bacillus virulent for Guinea-pigs.*

(*a*) *By passage through animals.*

An account of the experiments which have been made with the purpose of attempting to convert Hofmann's bacilli into diphtheria bacilli by means of passages through birds or otherwise has already been given (p. 219).

Apart from these experiments, several observers have tried to impart virulence to Hofmann's bacillus by passages through animals. From one of Ohlmacher's experiments it might be concluded that Hofmann's bacillus could be rendered virulent by a single passage through a susceptible animal, for example a guinea-pig, but the experiments of Bergey (1898, p. 46), Williams (1902), and others, distinctly show that even by successive passages through guinea-pigs Hofmann's bacilli cannot be rendered virulent to these animals. A passage through an immune animal, such as a rat, is also apparently incapable of producing virulence in this organism. The writer recently injected four strains of Hofmann's bacillus subcutaneously into white rats, and recovered them from the site of inoculation after 48 hours. The rats were unaffected and the cultures made from the organs remained sterile. Attempts to recover one strain after three days was unsuccessful. All the recovered strains were totally non-virulent to guinea-pigs in doses of 3 c.c. of 48 hour broth cultures. Two of these strains had been obtained from persons suffering from diphtheria-like diseases of the throat.

Fibiger (1895) was unable to raise their virulence by inoculation into guinea-pigs together with virulent streptococci.

(b) *By growth with other organisms.*

Bernheim (1894) was unable to make a Hofmann's bacillus virulent by continued cultivation together with streptococci.

(9) *Observations on the Changes in Morphological Type of Hofmann's Bacilli.*

(a) *After passage through animals.*

Films made from the seat of inoculation often contain many bacilli whose morphological characters do not entirely correspond with those of the organisms in the cultures from which the inoculation was made. In cultures made from the seat of inoculation, however, it is usually found that the organisms which grow are all of the same general morphological type as those in the original culture. Ohlmacher (1902) states that in one experiment a short, uniformly staining, Hofmann-like bacillus, after a passage through a guinea-pig, completely altered its morphological character in culture and became long and granular, and Hadley (1907) describes a D^3 type which "killed in 39 hours, with all the usual signs of diphtherial poisoning. When the organism was recovered it was found to be a barred variety." Williams (1902) on the other hand observed no changes in morphology in Hofmann's bacilli in cultures recovered from guinea-pigs after several successive intra-peritoneal passages through these animals, nor were any changes found after a sojourn of 48 hours in the body of an immune host (rat).

In a few experiments recently made with Hofmann's bacilli, obtained either from clinical cases of diphtheria or persons suffering from diphtheria-like diseases, the writer noticed no morphological changes in the cultures recovered from the tissues of inoculated rats.

(b) *In cultures.*

No observer appears to have noticed any changes in morphological type in Hofmann's bacilli grown on various culture media for considerable periods of time and then recultivated on serum. When grown on broth, media containing sugars, hard-boiled egg, and various other substances long, and even segmented, or very short types may be seen after a few days' cultivation. Transference of the organisms from such media on to serum invariably results in the culture exhibiting the ordinary Hofmann type, showing that no real change of type has occurred under these circumstances.

(10) *Evidence bearing on the Relationship of Diphtheria and Hofmann's Bacilli derived from Preventive Measures.*

A number of outbreaks of diphtheria, both limited and extensive, have now been recorded in which the preventive measures have included the examination of contacts and other possible carriers of the disease, and the isolation of such as have been found infected with diphtheria bacilli. In several instances (Chapter XII) these measures have been highly efficacious and have resulted in the complete, or almost complete, cessation of the disease, although persons harbouring Hofmann's bacilli have not been isolated or treated in any way. At the times when diphtheria is epidemic the conditions should prevail, if there are any, which are capable of turning Hofmann's bacilli into diphtheria bacilli. Yet in some of these outbreaks numbers of children harbouring Hofmann's bacilli, when separated from those harbouring diphtheria bacilli, have remained perfectly well without any cases of diphtheria amongst them. Even Wesbrook (1905), who in one outbreak met with diphtheria bacilli morphologically resembling Hofmann's bacilli, states in his most recent publications that in dealing with outbreaks the bearers of all other forms of diphtheroid bacilli than types A, C, D may be safely neglected.

These facts, so far as they go, indicate that Hofmann's bacilli under conditions which seem most suitable for the exaltation of their virulence, if they are really attenuated diphtheria bacilli, are incapable of giving rise to diphtheria.

The Opinions of recent Investigators on the Relationship of the Diphtheria to the Hofmann's Bacillus.

In the previous sections the various observations bearing more or less directly on the relationship of the diphtheria to the Hofmann's bacillus have been quoted, and in this section the views of some of those who have worked at the subject within the last ten years[1] are given. Some of these observers have investigated the problem from several points of view, whilst others have made extensive inquiries into certain points which appeared to them of fundamental importance. Others have expressed opinions on the most superficial observations. The views of the latter have not been recorded.

Those who consider that by artificial means they have succeeded in converting one organism into the other naturally believe in their identity. Of these Hewlett and Knight (1897) and Richmond and Salter (1898) are the most prominent. On various grounds, including experiments and observations on successive cultures from patients, Simonin and Benoist (1898), Lesieur (1901), Levin (1901), Gorham (1901), Ohlmacher (1902), and Behring all regard these organisms as very closely connected, if not identical. Salus (1902) regarded Hofmann's bacillus as a diphtheria bacillus which had become saprophytic, but considered that no diagnosis of diphtheria should be given when it was found. Wesbrook (1900, 1905) seems to regard all of his types as variants of the diphtheria bacillus, but, nevertheless, considers that in dealing with epidemics the Hofmann type may be safely neglected.

On the other hand a large number of observers have either definitely expressed the opinion that the organisms are distinct species, or that, whatever their relationship, Hofmann's bacillus has no etiological significance in the causation of diphtheria. Others again have only investigated points of special importance in regard to relationship, and have shown that no evidence could be obtained in favour of any connection between the organisms. Amongst such observers are the following, none of whom appears to attach any importance to the finding of Hofmann's bacillus in cultures: Peters (1896), Neisser (1896), Prochaska (1896), Fraenkel (1897), Spronck (1897), Glucksmann (1897), Todd (1898), Franke (1898), Bergey (1898), Theobald Smith (1898), Preisich (1899), Garratt and Washbourn (1899), Lubowski (1900), Gromakowsky (1900), Cobbett (1901), Zupnik (1902), Williams (1902), Schabad (1902), Neumann (1902), Korchoune (1902), Gordon (1902), Schwoner (1903), Thomas (1904), Graham-Smith (1904), Lewandowsky (1904), Petrie (1905).

---

[1] The authority of Roux (1890), whose opinion justly carries great weight, has often been quoted in support of the idea that Hofmann's bacillus is related to the diphtheria bacillus. But this is not right, for his remarks on the pseudo-diphtheria bacillus were made in comparatively early days, when the importance of acid-production had not been generally recognised, and before Hofmann's bacillus had been clearly distinguished from the so-called non-virulent diphtheria bacillus. Indeed in speaking of the relationship of what he calls the pseudo-diphtheria bacillus to the true diphtheria bacillus, Roux distinctly said that the former produced acid. He, therefore, at that moment was not speaking of Hofmann's bacillus, which forms no acid, but of what is generally now called the non-virulent diphtheria bacillus. It is only when he goes on to speak of the great frequency of the pseudo-diphtheria bacillus, both in Paris and in a country village, that one believes that he must have been then referring to Hofmann's bacillus, for the non-virulent diphtheria bacillus is relatively uncommon. It is highly probable that he did not test the acid-production of all these bacilli, the importance of the test then not being recognised, and that he confused Hofmann's bacillus, which forms no acid out of glucose, and is a common inhabitant of the throat, with the much rarer acid-forming non-virulent bacillus which cannot be distinguished by its appearance from the true diphtheria bacillus, and included them both in the term pseudo-diphtheria bacillus.

Some of these investigators have expressed the opinion that Hofmann's bacillus is frequently associated with an uncleanly condition of the mouth and teeth, and its more frequent occurrence amongst the children of the lower classes lends support to this view.

A number of workers have expressed themselves as unconvinced by the arguments brought forward on one side or the other, and consider that the controversy cannot yet be regarded as settled.

Summary of Chapter VI.

Hewlett and Knight (1897) considered that by heating cultures of the diphtheria bacillus they had once succeeded in converting it into a Hofmann's bacillus, but failed to bring about the transformation on other occasions. Other subsequent observers have also failed to confirm their statements, and even Hewlett (1904) has recently been unable to succeed. Trumpp (1896) also thought that he had brought about the same transformation by successive passages through guinea-pigs together with a non-fatal dose of diphtheria toxin. Hewlett and Knight (1897) as well as Richmond and Salter (1898) described experiments in which Hofmann's bacilli were apparently converted into diphtheria bacilli, the former observers by prolonged cultivation, the latter by repeated passages through small birds. Neither of these experiments appears to have been successfully repeated, and even the virulence of Hofmann's bacillus for the goldfinch has been denied.

In view of the fact that these experiments were made several years ago, and have not been successfully repeated, and of the fact that much evidence has accumulated against the identity of the two organisms, great importance cannot be attached to these experiments, unless they can be confirmed by subsequent experimenters.

The results of the most recent and exhaustive experiments have shown that Hofmann's bacillus produces no toxoids in culture, and moreover animals cannot be immunised against diphtheria bacilli by successive inoculations with cultures of this bacillus.

Again the capacity for producing acid out of certain sugars is confined to the diphtheria bacillus, Hofmann's bacillus being incapable of producing acid out of any of the substances which have been tested.

The evidence of agglutination experiments also decidedly points against the connection of the two species.

The general conclusion from these experiments, which have the most direct bearing on the relationship of the two organisms, is that no sufficient evidence has been produced in favour of their identity. In fact the most recent observations show, so far as they can, that these bacilli belong to distinct species.

Very numerous other observations and experiments have been made which have some bearing on this question.

Experiments have been quoted which prove that the majority of the morphologically typical diphtheria bacilli, which are found in healthy persons exposed to diphtheria, are fully virulent, and that they retain their virulence for long periods; in many instances up to the time of their final disappearance. Very little evidence can be produced for the statement, which is so frequently made, that during prolonged persistence in the throat diphtheria bacilli gradually lose their virulence. Experimental attempts to artificially decrease the virulence of diphtheria bacilli produce somewhat conflicting evidence, but show that under any conditions their attenuation is a matter of difficulty and uncertainty. Attempts to give virulence to totally non-pathogenic diphtheria bacilli have been almost uniformly unsuccessful, and even attempts to raise the virulence of lowly virulent bacilli have frequently failed. There is also no evidence to show that the virulence of diphtheria bacilli is altered by transference from one individual to another, or when different parts of the same individual are affected. Whatever the final verdict may be as to the relationship of the virulent diphtheria bacillus to the non-virulent form, no inferences can be made from it as to the relationship of the diphtheria bacillus to the Hofmann's bacillus.

It has frequently been asserted that diphtheria bacilli during convalescence gradually change their morphological type and become converted into Hofmann's bacilli, but many of the investigations on this subject are for various reasons of doubtful value, and the assertion has certainly not been conclusively proved. In fact there is much evidence to show that such a change does not take place. Changes of morphological type do not seem to occur by transference from one individual to another.

According to some observers prolonged cultivation is apt to change the morphological type, according to others it is not. In regard to changes of morphological type by passages through animals the statements are conflicting.

On the whole these experiments and observations also point to the conclusion that diphtheria and Hofmann's bacilli are not related to one another.

It has been asserted on various occasions that Hofmann's bacilli may be pathogenic to man, but no bacteriological proof has been produced, and, except by one or two observers, Hofmann's bacilli derived from all sources have been found to be non-virulent to guinea-pigs in fairly large doses. Attempts to give virulence to this organism or to change its morphological type have mostly been unsuccessful. Further the evidence derived from the bacteriological control of outbreaks of diphtheria, and the fact of the very general distribution of this organism are against the etiological significance of Hofmann's bacillus in diphtheria. Finally most modern investigators appear to regard diphtheria and Hofmann's bacilli as belonging to different species.

An examination of even the most recent writings on the bacteriology of diphtheria shows that many workers are still content to found far-reaching conclusions on the morphology in culture alone. Some have not even gone so far but have based their statements on the direct examination of smears. In not a few of these papers it is evident that the writers are scarcely aware that the identification of bacteria depends on anything else than their morphological characters, and such important aids to classification as their action on sugars and the effects of animal inoculations are often not even mentioned. In fact it is not too much to say that much of the work that is published on problems apart from routine diagnosis is absolutely valueless. Some of the conclusions may be perfectly correct, but the statements are advanced without proofs, such as would be required in any other branch of bacteriology. Inquiries which have for their subjects such important questions as the occurrence of diphtheria bacilli in persons not recently exposed to diphtheria, their occurrence in apparently non-diphtheritic lesions, in unusual situations, in milk, animals, food-substances, dust or soil, require that the identity of the bacilli should be proved by every possible means, including the inoculation of guinea-pigs, both with and without antitoxin. Yet on all these subjects papers have been written without the mention of any experiments to confirm the diagnoses founded on morphology. On such points as these the unconfirmed morphological diagnosis of even the most experienced observers cannot be accepted as proof of identity.

Whilst it is highly desirable that those who make routine bacteriological diagnoses should from time to time test the accuracy of their opinions founded on morphology by isolating and thoroughly examining bacilli from doubtful cultures, the necessity for this procedure is not so great as in work which is of the nature of research. In the former case no doubt a few patients suffer from inaccurate diagnoses, and the errors of the observer are likely to become more numerous as time goes on, but in the latter persons unacquainted with bacteriological methods are apt to accept unjustifiable conclusions on the prevalence of diphtheria bacilli to the great detriment of bacteriological preventive measures.

Even when every means of identification have been used by practised observers conflicting statements are common as exemplified in this chapter. Many of these are probably due to the unfortunate confusion which has prevailed owing to the wide range covered by the term "pseudo-diphtheria" bacillus.

There can be no doubt that Hofmann's bacillus is a common inhabitant of the throats and noses of healthy persons, at any rate in this country. Moreover numerous experiments have clearly demonstrated that from whatever source they are derived they are non-pathogenic for guinea-pigs in ordinary doses. Further evidence is rapidly accumulating to show that many other species of diphtheria-like organisms are to be found in the throat, nose, eye, genital tract, and on the skin of healthy and diseased persons, some of which more closely resemble the diphtheria bacillus and others the Hofmann's bacillus in morphology. "Since there are so many different forms or varieties of diphtheria-like bacilli it is quite possible that some of them are so closely related to the diphtheria bacillus that under certain conditions they readily develop its characteristics. This seems to be the only way to explain the apparent discrepancies in the results obtained by different observers" (Williams, 1902, p. 107). Such closely related varieties, if they exist, appear to be very rare.

Whatever the ultimate conclusion on this point may be, it is evident that much of the work dealt with in almost every section of this chapter might be advantageously repeated and extended. Opportunities constantly occur to those who are engaged in this subject of testing the accuracy of some of the observations. Many of these points are best worked out by very thorough experiments on a small scale, including all the necessary procedures for testing virulence under constant conditions, acid production, etc. In the case of inquiries into the change of type during long persistence in the throat or nose the influence of contaminating bacteria and other disturbing factors ought to be eliminated by the preparation of pure cultures.

Even if it is eventually proved, contrary to the opinion here expressed, that certain strains of Hofmann's bacilli represent saprophytic diphtheria bacilli, capable under artificial conditions of regaining virulence, this fact would not materially affect the conduct of bacteriological preventive measures unless proof was forthcoming that the change could also occur under natural conditions.

CHAPTER VII.

EXPERIMENTAL AND NATURAL DIPHTHERIA IN ANIMALS.

The lesions of experimental diphtheria in guinea-pigs, rabbits, dogs, rats and mice. Experimental and natural infection in cats, cows and horses. Diphtheria bacilli in the blood and organs of experimental animals. Diphtheria-like bacilli found in animals. Experimental lesions in birds. Observations on the relationship of avian to human diphtheria. Holmes' observations. Summary.

I. THE LESIONS OF EXPERIMENTAL DIPHTHERIA IN GUINEA-PIGS.

(a) *Macroscopic Lesions.*

SUBCUTANEOUS inoculations of pure cultures of diphtheria bacilli into the abdominal walls of guinea-pigs cause death usually within three days, and in typical cases result in the following gross anatomical lesions.

On removing the skin a grayish, necrotic, membranous focus is found at the seat of inoculation, frequently surrounded by a red zone of varying size. Underlying this area the abdominal wall is greatly congested. The subcutaneous tissue on the inoculated side is oedematous. The oedema may be slight, limited to a small area round the site of inoculation, or may extend over the entire side, and even into the neck, and in some cases over the median line to the opposite side. The oedema almost invariably assumes a *gelatinous* appearance, in some cases composed of pure yellowish serum and in others of serum stained with blood. The subcutaneous lymph glands of the axillary, inguinal, and cervical regions are usually haemorrhagic and swollen, the swelling being greatest on the side of inoculation. The pleural cavity frequently contains a considerable quantity of straw-coloured clear fluid, sometimes amounting to 12 c.c. or more. The peritoneal cavity occasionally contains similar fluid, but less frequently and in smaller quantity.

Both the visceral and parietal layers of the peritoneum are usually injected and ecchymoses are present in many cases. The *suprarenal glands* always show intense congestion and in some instances may be haemorrhagic, the colour of the organs varying from pink to a very dark red.

These are the principal lesions encountered in experimental diphtheria in guinea-pigs. Other gross lesions are, however, frequently met with. The lungs often show areas of congestion, and even consolidation. The liver is generally congested and often fatty. It occasionally shows on its surface macroscopic lines and dots, varying in size from a pin's point to a pin's head. Though the spleen is not perceptibly enlarged in all cases, yet in some. it is markedly enlarged, and the kidneys are frequently hyperaemic.

The mesenteric, retroperitoneal, mediastinal and bronchial glands are very frequently enlarged and often reddened, and the agminated glands of the coecum and ileum are generally abnormally prominent.

(*b*) *Histological Lesions.*

The seat of inoculation. Bacilli are to be found in the necrotic focus both free and in leucocytes, but they do not usually occur in the oedematous fluid at a distance from the focus. Sections made from the seat of inoculation show the bacilli in great numbers, and those stained by Weigert's fibrin method exhibit in a striking manner the bacilli and fibrin.

Welch and Flexner (1891) summarise the local changes in the following sentences. "The local action of the bacilli is of a most intense character. There is emigration and great destruction of leucocytes shown by the disintegration of their nuclei; the fixed cells of the part have undergone a similar fragmentation, nuclei of connective tissue and muscle have succumbed, and leucocytes have wandered into these areas, many of the latter being also destroyed."

Axillary and Inguinal and other Lymph Glands.

A very minute account of the histological changes found in the lymph glands is given by Welch and Flexner (1891) who regard the changes as very typical: "There are haemorrhages under the capsule and into the substance of the gland. The blood vessels here, as elsewhere in the body, contain a greatly increased number of leucocytes. The cells of the glands are the seat of great changes. The principal lesions are in the lymph follicles, the lymph cords and lymph sinuses being also affected, though in less degree. Different follicles are affected in different degrees, but in no instances were the lesions entirely absent. The lesions consist of a marked alteration in the number, character, size, staining capacity and configuration of the nuclei making up the parts affected. The cell bodies are altered also, and an increased number of cells differing from the lymphoid type are found. The first thing that attracts attention is the unusual number of deeply staining bodies in the tissue. These bodies are observed to vary in size and shape, and under a sufficiently high power some of them are recognised as nuclear figures.

They are usually however globular, and under a magnifying power of four hundred, range from fine dust-like particles to larger particles appearing with this power the size of a pin's head. The finer particles are often aggregated into globular masses, which are now free and now enclosed in cells. There are, again, deeply staining particles present, which show decided bizarre forms. Imperfect crescents, flask-shaped, bladder-like, whetstone, angular, and dumb-bell forms are more or less common. Occasionally, nuclei appear as if one end were drawn out and constricted into a ball-like protuberance that is being pinched off. The globular particles are, at times, grouped together with the bizarre forms into larger masses; what particularly distinguishes these bodies from the normal nuclei which remain is the intensity with which they stain.

All the chromatin particles, as before mentioned, are not within cells; indeed, as a rule, they do not occur in cells, though the number within cells varies considerably. In some glands much of this material is contained within globular cells several times larger than the lymphoid cells. In some sections these cells are observed to be present in considerable numbers, partly devoid of stained particles, or nearly so, but usually they are full of nuclear detritus.

In certain spots there is almost an absence of stained cells and particles. In these places it is possible to distinguish outlines of cells, then a finer, granular, somewhat refractive, at times reticulated, material, and here and there a deeply stained particle. But there are always a few cells remaining that stain more or less, and amongst these cells are round cells, larger than the lymphoid cells. The portion stains with Weigert's fibrin stain in such a manner as to indicate that the granular material is largely made of fibrin or of a substance allied to fibrin. There occur in the lymph sinuses more especially a considerable number of round, or slightly oblong, cells, quite colourless in appearance, containing red blood corpuscles."

"An alteration similar to that just described is found throughout the lymphatic structures of the body: in the spleen, mesenteric glands, retroperitoneal glands, intestinal lymphatic apparatus (Peyer's patches, solitary follicles, and diffused lymphatic tissue), the bronchial glands, mediastinal glands, and cervical glands. The only variation is one of degree. As far as our study has gone every gland examined has been more or less affected. The *Spleen* is often very rich in nuclear fragments and foci of coagulation necrosis."

Suprarenal Glands.

The blood vessels are congested and haemorrhages are frequently present. Occasionally the medullary cells are distinctly hyaline (Welch and Flexner, 1891).

Lungs.

There are often haemorrhages under the pleura, and all the blood vessels of the lungs, including the capillaries, are often distended with blood, and even haemorrhages into the alveoli may be found. An exudation of leucocytes and fibrin may occur into some of the alveoli. Fragmentation of the nuclei, except in the epithelial lining of the larger bronchi, is not common in the lungs (Welch and Flexner, 1891).

Liver.

In sections of the liver the dots and lines already mentioned are found to be areas of dead hyaline liver cells. Leucocytes are commonly found in such foci. These appearances were first observed independently by Welch and Flexner (1891) and Dubief and Bruhl (1891) about the same time. Welch and Flexner (1891) called attention to intralobular haemorrhages due to rupture of the walls of the central veins of certain lobules, and stated that the walls of these vessels, which were quite refractive in the fresh state, showed after hardening a characteristic hyaline appearance.

Kidneys.

Fatty changes are found in the epithelium of the tubules and glomeruli of the kidney. Welch and Flexner (1891) laid stress on the hyaline alteration of the glomerular capillaries and smaller arteries, and observed that a hyaline substance completely filled the lumen of some capillaries.

Heart.

Fatty degeneration is commonly found in the heart muscle. According to Flexner (1894) this depends more on the intensity of the poison than on the time of its action. Mollard and Regnaud (1897) found various degeneration changes, such as alteration or disappearance of striation and hyaline changes, as well as interstitial sclerotic lesions in the heart muscle of guinea-pigs dying 17 days after inoculation.

Vessels.

Mollard and Regnaud (XI. 1897) occasionally met with endarteritis in chronic cases. The same authors (VII. 1897) once met with atheroma of the aorta in a guinea-pig dying eight months after the injection of dilute toxin.

Muscles.

Abbott and Ghriskey (1893) noticed in sections made through the seat of inoculation that many of the muscle fibres were in a condition of hyaline degeneration and were here and there invaded by diphtheria bacilli. Sometimes the penetration would occur through the side of the fibre, sometimes from one end (Pl. XIII, fig. 3).

Brain.

Degeneration of the cells of the brain has been noticed, especially in prolonged cases, by Berkley (1897) and others.

Intestines.

Welch and Flexner (1891) carefully studied the intestinal lesions. The cells of the villi and their epithelium showed lesions which varied in intensity. "The most striking changes consisted in a fragmentation of the nuclei of the cells in the villi, especially of those surrounding the central vessels, a disappearance of a large number of cells and the presence of large round cells, similar to those described in the part of the lymph glands most affected. These larger cells often showed a very slight staining power, and shadows of cells were not uncommon. The nuclei of the epithelium were distinctly and extensively fragmented. Nuclear figures were to be seen and saprophytic bacteria were found in the necrotic tissue. These fragmented nuclei partook of the same characters as those already described, and exhibited the same intense affinity for staining agents."

Cells in the pleural effusion.

Courmont and Arloing (1901) found that the cells in the pleural effusion in most guinea-pigs consisted almost entirely of various forms of lymphocytes. In one animal only were polynuclear cells present to the extent of 25%.

The writer has recently made differential leucocyte counts in films made from the deposit obtained by centrifugalising pleuritic effusion, which do not bear out the above statement. In ten examples the proportion of the various cells in each case was nearly identical.

Hyaline cells and lymphocytes	44·99%.
Finely granular eosinophile cells	2·06%.
Coarsely granular eosinophile cells	52·93%.

Leucocytes.

The number of leucocytes in the blood is increased (Park, 1900).

Intraperitoneal inoculation.

Intraperitoneal inoculation produces similar changes to subcutaneous inoculation, except for the absence of subcutaneous lesions. At the autopsy the inflammatory manifestations in the peritoneum are more marked. Roux and Yersin (1889) were the first to point out that with intraperitoneal inoculations these animals take longer to succumb, than when subcutaneous injections are given.

PLATE XIII.

Fig. 1. Area of necrosis at the seat of inoculation in cow No. 1 (p. 285), showing the condition of fragmentation of the cell nuclei and clumps of irregularly stained *B. diphtheriae*. Stained with Bismarck brown and Gram's method. (In the original plate the bacilli appeared violet, the tissues brownish-yellow, the nuclei etc. darker brown.) Leitz $\frac{1}{12}$, Oc. 4. (From Abbott (1893), Plate, fig. 1.)

Fig. 2. Section through focus of leucocytes located in the omentum of a guinea-pig between the layers of the peritoneum. Leitz Obj. 3, Oc. 1.

Fig. 3. Hyaline muscle fibres from the seat of inoculation showing penetration of the bacilli into them. Stained with eosin and methylene blue. Leitz Obj. 7, Oc. 3.

Fig. 4. Contents of one of the foci (Fig. 2) dried upon a cover-slip and stained with Loeffler's blue. Leitz $\frac{1}{12}$, Oc. 3.

(Figs. 2, 3 and 4 are from Abbott and Ghriskey (1893), Plate, figs. 1, 4 and 2. Figs. 2 and 3 in the original are stained red and blue, and fig. 4 blue.)

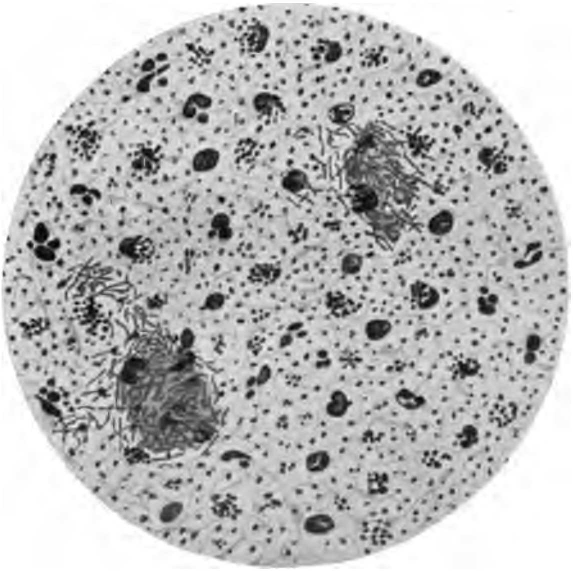


Fig. 1.

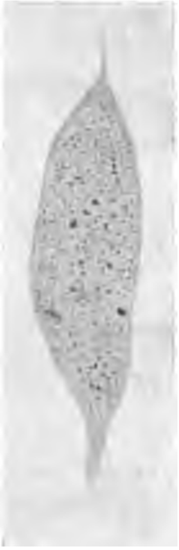


Fig. 2.

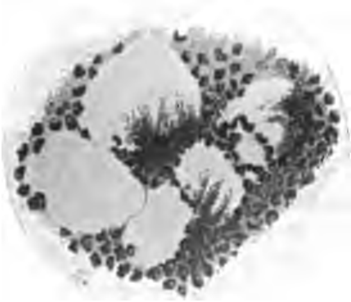


Fig. 3.

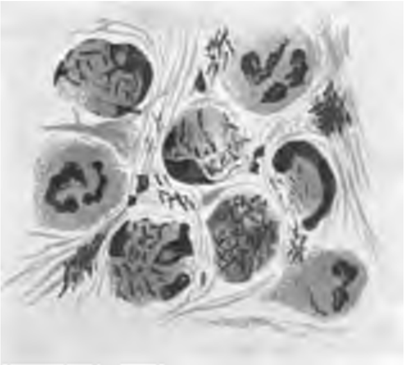


Fig. 4.

Tracheal inoculation.

By inoculating the abraded mucous membrane of the trachea with cultures a pseudo-membrane can frequently be produced. Roux and Martin (1894) observed the extension of such membranes down to the primary ramifications of the bronchi. Belfanti (1896) produced only bronchopneumonia by the injection of cultures into the uninjured trachea, but similar experiments by Henke (1898) gave negative results.

Inoculation of mucous surfaces.

Loeffler, Roux and Martin (1894) and many later observers have produced pseudo-membranes by the inoculation of the abraded mucous membrane of the vagina. Henke (1898) observed that sometimes oedema alone without membrane was produced. These pseudo-membranes resemble histologically those of the human subject.

Cornea.

Pseudo-membranous inflammation may be produced by rubbing cultures into the abraded surface of the cornea.

Inoculation into the testes.

Abbott and Ghriskey (1893) were the first to describe certain lesions of the omentum, which could be only produced with certainty by the injection of cultures into the testes. The lesions consist of minute yellowish, lens-shaped foci located in the omentum, usually between its peritoneal layers, and are most common near the free margin. When visible to the naked eye they are rarely larger than the eye of a small cambric needle and of about the same shape. The histological structure of these nodules is simple, the foci consisting of polynuclear leucocytes, the majority of which contain diphtheria bacilli (Pl. XIII, figs. 2, 4).

"A point of particular interest in connection with the cases in which the injection had been made into the testicle was the diminution of oedema of the superficial tissues, and its increase in the tissues of the abdominal cavity. In two of the cases straw-coloured fluid was not only found free in the peritoneal cavity, but the small intestine was practically filled with fluid and its walls were intensely oedematous."

Paralyses.

Various paralyses accompanied by lesions of the nerves and cells of the central nervous system may be produced in guinea-pigs and other animals by the injection of cultures simultaneously with or followed by antitoxin, or by the injection of small doses of toxin. The paralytic phenomena are dealt with later (Section V).

II. THE LESIONS OF EXPERIMENTAL DIPHTHERIA IN RABBITS.

Guinea-pigs have been almost universally used to determine the identity and pathogenic powers of diphtheria bacilli. Rabbits are, however, susceptible to inoculations of diphtheria bacilli and their toxins, though life is more prolonged than in the case of guinea-pigs. These animals have, however, been used for the study of various lesions.

Time of Death.

Death may occur in four days with the injection of virulent cultures, or life may be prolonged for months when small doses of old cultures are given.

Leucocytes.

Schlesinger (1902) studied the blood cells in rabbits inoculated with virulent cultures. He observed that two hours after injection there was a hypoleucocytosis, but later a hyperleucocytosis.

Subcutaneous inoculation.

The animals die in four days or more, depending on the quantity injected. The following lesions are found at the autopsy: *subcutaneous oedema* is always present and is often very extensive, and sometimes it is haemorrhagic. The axillary and inguinal glands are swollen and the histological lesions are found to be the same as in guinea-pigs.

The *liver* is friable and yellowish with fatty degeneration (Roux and Yersin, 1888). In one instance Welch and Flexner (1891) found an extensive diffuse fatty degeneration of the liver tissue. The cells stained very imperfectly, many not at all. The nuclei of the liver cells were in many places in a state of disintegration or fragmentation; a few of division. The cells throughout entire lobules were affected.

The *lungs* are usually not affected, and exudation into the pleura is exceptional.

The *kidneys* generally show fatty changes in the epithelium of the tubes and glomeruli.

Fatty degeneration of the *heart* is very common. Mollard and Regnaud (1897) observed well-marked atheroma of the aorta in one instance in a rabbit which died six months after the injection of toxin.

There is usually much congestion of the *mesentery* and *omentum*, and ecchymoses are frequently present. The *intestinal villi* show the same changes as in guinea-pigs.

Trambusti (1896) investigated the *marrow*, and found that in the early stages there was an unusual amount of cell division, but as the disease progressed degenerative changes were found in the giant cells and leucocytes.

Crocq (1895) made careful investigations on the *nervous lesions* occurring in rabbits inoculated with four months' old cultures, under which conditions the animals lived for periods varying from one to three months. He states that lesions are found in the cord and nerves, rarely in the medulla, and never in the brain. These lesions consist of swelling of the nerve cells, loss of their protoplasmic prolongations, disappearance of nuclei, and extensive proliferation of the cells of the ependyma. Acute myelitis is rare. The lesions of the nerves appear from the fifth day onwards. The axis cylinder segments, the protoplasm becomes more abundant, the myelin becomes granular and is absorbed, and finally the axis cylinder disappears. Of the nerve roots the anterior alone are affected. In the rabbit it seems that the cord lesions precede those of the nerves.

Intravenous inoculations.

Loeffler observed that death did not invariably follow intravenous inoculations, but Roux and Yersin (1888) found that all the animals thus treated died in about 60 hours with a dose of 1 c.c. Metin (1898) and others have found that intravenous injection is usually fatal.

At the autopsy Roux and Yersin (1888) found general congestion of the abdominal organs, dilatation of the vessels, swelling of the glands, marked nephritis, and fatty degeneration of the liver, which had a yellow tinge. In animals which did not die so rapidly typical paralyses followed.

Meyer (1902) produced endocarditis of the injured valves by direct injection of diphtheria culture into the heart.

Tracheal inoculation.

Loeffler, Roux and Yersin (1888), Bardach (1895), Henke (1898), and others have found that inoculations with cultures onto the abraded tracheal mucous membrane after preliminary tracheotomy produce pseudo-membranes. The disease so produced is almost always fatal. The rabbits breathe with difficulty, and the membrane is apt to spread from the trachea to the fauces. Oedema of the tissues and enlargements of the glands of the neck are constantly present. Henke (1898) states that the pseudo-membrane consists of a fibrinous network, enclosing in its meshes leucocytes and cast-off epithelium. In the place of the normal epithelium a cloudy layer is seen representing it. The submucous layer is infiltrated with leucocytes and shows exudation and haemorrhage. Bacilli are found in the membrane but not in the submucous layer.

Flexner and Anderson (1898) made very extensive investigations on the introduction of cultures through tracheotomy wounds without further injury to the mucous membrane. Only small patches of congestion occurred in the trachea, but the lungs were often completely consolidated and presented a peculiar semi-gelatinous appearance. On section oedematous fluid escaped in small quantities. In several cases cultures from these lungs were negative, but others yielded diphtheria bacilli. In some instances, however, pneumonic processes in the lungs were not provoked.

Cultures made from the lungs of rabbits killed 1, 3½, 6, 12, and 18 hours after injection showed bacilli, but cultures from animals killed 24 hours after inoculation remained sterile. Their examinations proved that the bacilli were rapidly taken up by the cells, but not by the polymorphonuclear leucocytes.

Lack (1899) was able to produce a pseudo-membranous inflammation of the trachea in a rabbit with a culture derived from a case of fibrinous rhinitis.

Stecksen (1900) was unable to produce pseudo-membranes with killed cultures.

Conjunctival inoculation.

Babes (1890) experimented with a number of rabbits and found that inoculation of the conjunctiva with cultures gave rise to thick exudates in 24 hours. Some of the animals died in eight to 15 days, and some developed paralysis and eventually died. The exudate consisted of a fibrinous material containing fragments of cells and a few bacilli, and histologically resembled human diphtheria membrane. Several subsequent observers have confirmed the observation that pseudo-membranes may be produced by inoculations onto the injured cornea and conjunctival mucous membrane.

III. THE LESIONS OF EXPERIMENTAL DIPHTHERIA IN DOGS.

Dogs are susceptible to the action of the diphtheria bacillus and its products, but they have not been very extensively used for experimental purposes. The principal lesions produced by the subcutaneous injection of cultures are the same as in rabbits, extensive oedema, fatty lesions of the heart, etc. Lesions of the nervous system seem to be very easily produced in these animals, both by the injection of toxins and of living cultures. Roux and Yersin (1889) record experiments on dogs in one of which a dog died stupid and paralysed three days after subcutaneous inoculation, and in another in which the animal died in the same condition four days after tracheal infection. No pseudo-membrane was found in the trachea at the autopsy.

IV. EXPERIMENTAL DIPHTHERIA IN RATS AND MICE.

It has long been known that rats and mice are extremely resistant to diphtheria bacilli and their toxins. Unless enormous doses are injected the animals are not affected. Roux and Yersin (1888, p. 661), for example, inoculated a mouse subcutaneously with ·3 c.c. and another with 1 c.c. and two others with 2 c.c. of a filtrate, which was capable of killing a 350-grm. guinea-pig in a dose of ·2 c.c. None of the animals were affected in any way. A young rat was inoculated with 2 c.c., also without any ill effect. Cobbett (15. IV. 1899) made some experiments to test the relative resistance of rats and guinea-pigs to diphtheria toxin. He concluded that "the white or black rat of 100 grams weight is only relatively insusceptible to the action of the products of the diphtheria bacillus, and succumbs to the subcutaneous injection of filtered cultures, in quantities which are, weight for weight, from 1500 to 1800 times as great as those which suffice to kill guinea-pigs of 250 grms.[1]" The tissues are but little affected locally by the injections of large quantities of filtrate, and have not been observed to suffer necrosis. These results are not due to the presence of anti-toxin in the blood, as it has never been found in the blood of normal rats.

---

[1] Goodman (15. VI. 07) finds that it requires 3500 times the guinea-pig lethal dose to kill full-grown rats.

Roux and Yersin (1888) found that to kill a mouse it was necessary to inject 80 times the fatal dose for a guinea-pig, and that rats also resisted very large quantities.

Some of the writer's recent experiments with living cultures gave the following results. Out of five rats subcutaneously inoculated with large doses of virulent cultures, one died in four days with a collection of pus in the right pleural cavity and another between the liver and the diaphragm. Cultures from these abscesses yielded pure growths of the diphtheria bacillus. In another rat killed 120 hours after inoculation a small abscess containing diphtheria bacilli was found. The others were killed 48, 72, and 168 hours after injection respectively, but no lesions were discovered. From the first of these animals cultures were obtained from the site of injection, but not from the others. Rats inoculated with non-virulent diphtheria bacilli and Hofmann's bacilli showed no lesions. From some of these animals killed 48 hours after inoculation cultures could be obtained from the site of inoculation, but not from those killed later. The blood and organs in all cases were sterile.

v. Hibler (1896) calls attention to, and illustrates the presence of large numbers of diphtheria bacilli in the cells of the peritoneal exudate in mice five hours after the injection of diphtheria bacilli.

No instances of natural infection in guinea-pigs, rabbits, dogs, rats or mice have been recorded.

V. EXPERIMENTAL DIPHTHERIA IN CATS.

Klein (1888) produced diphtheritic lesions in cats by rubbing fresh diphtheria membrane onto the abraded surfaces of the cornea and palate. In 24 hours the parts became congested. After 48 hours the conjunctiva was greatly swollen, and the eye closed, and a muco-purulent material flowed from it. The cornea was opaque, and the palatine arch covered with a thin whitish-yellow film. After 72 hours the oedema and swelling had increased and the cornea was ulcerated and covered with an adherent membrane. The palate symptoms increased up to the 10th day and then gradually subsided. The eye symptoms only began to subside in two or three weeks. Eventually both lesions entirely healed. A second series of cats inoculated from the first went through the same course of events, with the exception of one which died on the 4th day. Similar lesions were produced in cats by the application of diphtheria cultures to the abraded surface of the conjunctiva (1889, p. 157).

Subcutaneous inoculation.

Klein (1889, p. 165) made several observations on the effects of subcutaneous inoculations of diphtheria cultures in cats. Six cats were inoculated in the groin and all showed considerable swellings on the 3rd day. Two died on the 5th day, two on the 10th day and two on the 11th day.

The autopsies in all cases showed extensive subcutaneous haemorrhagic oedema, and an almost gangrenous condition of the tissue in some places. The spleen and liver were only slightly congested, but the condition of the *kidneys* was remarkable. These organs were enlarged and the medulla congested, but the cortex was gray or yellowish-white in colour. Under the microscope the cells of the renal epithelium, especially those of the convoluted tubules, showed extensive fatty degeneration and breaking down into granular débris. Seven other cats similarly inoculated died between two and seven days after injection, and showed the same changes. Two cats inoculated simultaneously on the cornea and subcutaneously in the groin showed marked eye changes, with only slight signs on the groin. When killed after 19 days only slight renal changes were found.

Tracheal inoculations.

Broth cultures injected directly into the trachea by means of a syringe pushed through the anterior wall caused death in one animal in 26 hours, and another became paralysed in the hind limbs and very ill on the 6th day. The autopsy on the second animal showed a bronchopneumonic condition of the lungs, enlargement and fatty degeneration of the liver, and enlargement of the kidneys. The cortex of these organs was a uniform whitish-yellow mass. In microscopic sections of the lung characteristic diphtheritic false membranes were found in the bronchi and fibrinous exudation in the infundibula. Diphtheria bacilli were cultivated from the lungs (Klein, 1889, p. 159).

Welch and Abbott (1891) found that the application of cultures to the abraded tracheal mucous membrane in kittens produced typical diphtheritic false membranes in the trachea. The animals died in a few days.

Feeding experiments.

Klein (1890, p. 229) fed two cats on several occasions with milk mixed with diphtheria bacilli. The animals became thin, but showed no prominent symptoms. They were eventually killed after three and four weeks respectively. Both showed greatly enlarged livers and large kidneys with a white cortex, and the lungs of one showed patches of gray hepatisation. Klein remarks that "from this experiment it is seen that by repeated feeding with cultures of the diphtheria bacillus distributed in milk, unquestionable diphtheria disease can be produced in the cat."

According to all Klein's experiments a fatty condition of the cortex of the kidney is very characteristic of diphtheria in cats.

Accidentally acquired diphtheria.

A cat placed in a cage with another, which had been inoculated on the cornea, also developed a conjunctivitis which was less severe, but resulted in ulceration (Klein, 1889, p. 158).

During some of Klein's (1889, p. 174) experiments on cows (p. 283) the cats in a certain shed were fed by mistake on milk containing diphtheria bacilli. In this shed a series of animals became ill during a period of two months, though healthy when procured. They suffered from an acute catarrhal affection of the conjunctiva and respiratory passages. Sixteen animals in all were affected and seven died after becoming much emaciated. At the autopsies the prominent pathological changes were lobular pneumonia, with fibrinous exudation containing diphtheria bacilli in the bronchi and infundibula, and enlarged kidneys with fatty degeneration of the cortex. In one animal there was a false membrane in the larynx and trachea in which diphtheria bacilli were found.

Nine months (1900, p. 235) later a similar outbreak occurred, but the source of infection was not found. In those animals which died the gross anatomical changes were similar to those just described, and in one there was a membrane in the larynx and trachea containing morphologically typical diphtheria bacilli.

The natural disease in cats.

A general impression prevails that cats contract diphtheria from human subjects, and very numerous instances are quoted in the literature[1] of cats, apparently suffering from diphtheria, communicating it to children, and other examples are cited of cats, although remaining quite well, being the only probable carriers of infection. Very few of these cases seem to have been bacteriologically examined, and in no instance has the presence of diphtheria bacilli been satisfactorily proved.

---

[1] Instances are given in considerable detail in the following papers:—Turner, G., *Local Government Board Report*, Vol. XVI. p. 309, 1886. Bruce-Low, *Ibid.* Vol. XVIII. p. 131, 1888. Letters by Drs Downes, Shirley-Murphy, and Thersfield quoted by Klein, *Ibid.*, Vol. XIX. p. 163, 1890. Symes, J. O. (6. VI. 1896), *Brit. Med. Journ.*, Vol. I. p. 1385, and Gray, H. (31. III. 1896), *Journ. of Comp. Path. and Therap.* Vol. IX. p. 46.

Klein (1889, p. 162) made autopsies on several occasions on cats which were regarded as carriers of the disease. One had become paralysed, and all showed the lesions which he described as characteristic of experimental diphtheria in cats. Diphtheria bacilli were not, however, cultivated from any of them.

Dowson (1895) records the examination of a cat which "was undoubtedly suffering from diphtheria." This animal became ill 14 days after a child in the house had died of diphtheria. It was killed on the 8th day of the disease. The autopsy showed the lungs to be in a condition of disseminated pneumonia, but all the other organs were healthy including the mouth, nares, larynx, and trachea. The kidneys were not examined. Cultures made from the lungs gave pure cultivations of an organism morphologically resembling the diphtheria bacillus, which was not, however, further investigated. In another cat, which he examined under similar conditions, the kidneys were found to be in the condition of fatty degeneration described by Klein, and the lungs showed some suppurating areas. From the latter, amongst other organisms, a few colonies of bacilli resembling those found in the first cat were discovered.

As an example of a case in which a cat was the probable carrier of the disease, an interesting observation by Low (1888, p. 131) may be quoted: "A little boy was taken ill with what turned out ultimately to be diphtheria. On the first day of his illness he was sick, and the cat which was in the room at the time licked the vomit off the floor. In a few days (the child meanwhile having died) the animal was noticed to be ill, and her sufferings were so severe and so similar to those of the dead boy that the owner had her destroyed. During the early part of its illness this cat had been let out at nights in the back yard as usual. A few days later the cat of a neighbour, who lived a few doors further off, was noticed to be ill. It had also been out in the back yard at night. This second animal, which, however, recovered, was the pet and playfellow of four little girls, who, grieved at the illness of their favourite, nursed it with great care. All four girls developed diphtheria, their mother being convinced they got it from the cat, and indeed no other known source of contact with infection could be discovered."

VI. EXPERIMENTAL DIPHTHERIA IN COWS.

Klein (1889, 1890) made several experiments on cows for the purpose of determining whether cows could be so infected with diphtheria bacilli that the latter made their appearance in the milk. The first experiments (1889, p. 168), simultaneously carried out on two cows, are quoted below at some length. Two healthy cows were chosen which had calved respectively three and four weeks previously. Each received under the skin of the left shoulder one hypodermic syringe full of broth culture of very virulent diphtheria bacilli incubated for three days at 37° C. On the third day a soft tender tumour about the size of an orange could be felt in each at the site of injection. The tumours increased in size for a few days, and then gradually decreased and became firm. The only constitutional disturbance noted during the first week was a slight rise of temperature during the 2nd and 3rd days in both cows.

On the 4th day a small vesicle appeared on the hind teat of cow No. 2, and others on the udder near to it. On the 5th day the vesicle had become covered with a brown crust, and the skin around was indurated. At this time there were on the udder several vesicles, some containing clear serum and others containing pus. All were slightly raised on a slightly injected corium. On the removal of one of the crusts an ulcer was exposed covered with a purulent film. The pustules and crusted ulcers differed in size; some measured not more than ⅛ of an inch in diameter, others measured up to ½ inch. The numbers of the vesicles had increased on the 6th day. In all cases there first appeared a vesicle filled with clear serum, which became a pustule, and later an ulcer covered with a crust, which gradually healed, the whole process occupying 6—8 days. From these lesions diphtheria bacilli were recovered in culture. A similar series of lesions occurred in cow No. 1.

Cow No. 1 became very ill by the 13th day and died on the 14th. At the autopsy a tumour about four inches square was found at the site of injection, consisting of laminae of fibrous tissue and necrotic material, and closely connected with the skin and underlying muscle. On section clear fluid oozed from the mass. The neighbouring lymph glands were enlarged, the upper lobes of the lungs and the kidneys congested, and the liver showed numerous gray necrotic patches. Cow No. 2 became ill and was killed on the 25th day. The lesions found at the autopsy were very similar.

'Microscopic sections made from the substance of the tumour showed necrotic reticulated masses containing large and small aggregations of the diphtheria bacilli. These aggregations of bacilli extended into the muscular tissue nearest to the septa, in fact the implication of the muscular fibres by the gradual growth into them of the masses of bacilli could be easily traced. Further, careful microscopic examination of the masses of bacilli in the necrotic portions of the tumour, as also of those implicating the muscular fibres, revealed the following remarkable fact, namely, that many bacilli had become threads, some of considerable length, and containing granules in their course, characterised by intermediate or terminal buds or swellings, spherical, oval, or flask-shaped. In a word, the organisms had taken on forms, which are but exaggerations of what has been before observed in cultures of this bacillus." "That these threads in the tissue of the tumour had been really developed from the diphtheria bacilli was proved by the circumstance that all intermediate forms between the typical diphtheria bacillus and these hyphae-like threads could be everywhere seen in the same microscopic section: and as well by the fact that every one of the colonies in the cultures obtained from the tumour were found to be pure diphtheria bacilli."

On the 5th day of the experiment a teat of cow No. 1, which was still normal, was cleansed and scrubbed, and milk collected from it into sterile tubes with every precaution against accidental contamination. From this milk diphtheria bacilli were cultivated in small numbers (about 32 colonies per c.c.).

With scrapings taken on the 6th day from the eruptions of cow No. 2 two calves were inoculated in subcutaneous incisions of the belly and groin. On the 6th day after the inoculation red papules appeared, and on the 8th vesicles formed, and continued to appear for 18 days, going through the stages already described in the cows. The animals developed a muco-purulent discharge from the nose, and a cough, and showed difficulty in breathing, and they fed very little. The lung trouble increased, and they became thin, and were killed on the 25th day. The autopsies showed enlarged oedematous inguinal glands, general congestion of the lungs with consolidation of the upper lobes, and well marked fatty degeneration of the cortex of the kidneys.

Further experiments with four cows (subcutaneous inoculation of cultures) resulted in the death of the animals, and similar lesions were found at the autopsies as in the first animals, but the teats and udders remained normal and no diphtheria bacilli could be cultivated from the milk.

Another set of similar experiments with two cows (Nos. 7 and 8) resulted, however, in the formation of eruptions on the teats and udders similar to those in cows 1 and 2. From the milk of cow 8 diphtheria bacilli were isolated. Inoculations with old, rather attenuated, cultures of diphtheria bacilli only produced slight swellings and little constitutional disturbance.

Two other cows (Nos. 9 and 10) were subcutaneously inoculated with broth cultures, and in one an ulcer appeared on the base of a teat on the 8th day. Each cow had a sucking calf, both of which on the 9th day showed vesicles surrounded by red areas on the upper lip. In the next few days several vesicles developed round the earlier ones. No cultures appear to have been made from these lesions. No diphtheria bacilli were found in the milk of these cows on the 10th day after inoculation, *i.e.*, on the day subsequent to the appearance of the eruption on the lips of their calves. A calf subcutaneously inoculated with a virulent culture developed a large, soft, tender swelling over which the epidermis desquamated in large flakes. Cultures of diphtheria bacilli were obtained from the swelling.

Cutaneous inoculations.

Five calves were inoculated along incisions in the abdominal wall, two with cultures of diphtheria bacilli, and three with fresh human diphtheria membrane. In three days the incisions became tumid and red, and later the central portion developed a thin linear crust with indications of vesiculation at the edges. In those inoculated with cultures the condition subsided, but in those infected by means of membrane the condition went on to ulceration.

Feeding experiments.

Feeding experiments with broth cultures on two cows produced no results.

Abbott (1894) repeated some of Klein's experiments on the subcutaneous inoculation of cows, but did not confirm his results. One of the two cows he used was tuberculous and died in 16 days. No eruptions occurred on the teats, but a tumour developed at the site of inoculation. The tumour consisted of a peripheral zone of fibrous tissue and an internal necrotic mass containing muscle fibres and connective tissue in various stages of degeneration. In some parts there was only a reticulum of fibrin with fragments of cells. Although clumps of diphtheria bacilli could be seen in sections of the tumour none could be cultivated (Pl. XIII, fig. 1). The second cow was healthy and suckling a calf. She developed a swelling at the site of inoculation but no eruptions on the teats, and was killed on the 20th day. At the autopsy the lungs were found to be normal, but the liver showed three yellowish fatty patches and the kidney one small fatty area. The milk of both the animals was studied for nine days after the inoculation and all suspicious bacilli were isolated and tested, but no diphtheria bacilli were found. Abbott consequently thought that diphtheria bacilli did not pass into the milk as described by Klein. Ritter (1896) also attempted to repeat Klein's experiments, but failed to show that the diphtheria bacilli passed into the milk.

Klein (1894) replied to Abbott's criticisms, and raised certain objections to his experiments and deductions. He showed that Abbott had only seen the accounts of his first two experiments and not those of the remainder which confirmed them, and argued that the animals used by Abbott were not fit subjects for the experiment, in that one was tuberculous and the other in poor condition. He also considered that the cultures used were unsuitable, since they were not very virulent as proved by Abbott's experiments with them on guinea-pigs, the slight reaction produced in the cows, and his inability to grow them from the local lesion.

Spontaneous infection in the cow.

Thorne-Thorne (1891), in reviewing the chief epidemics of milk-diphtheria in England, sums up as follows: "On each occasion of milk-diphtheria to which I have referred there has been some evidence, more or less precise, of some cow ailment, so far trivial, it is true, as to be ignored by those versed in bovine diseases, but either affecting the physical properties of the milk, or being associated with some vesiculation and later on with 'chapping' or 'scabbing' of the udder and the teats."

Klein (1890) after his experiments pointed out that in two outbreaks of diphtheria, one near Croydon and the other near Bishop's Stortford, lesions occurred in the udders of the suspected cows, having the same characters as those produced by experimental inoculation. He did not claim, however, to have demonstrated bacteriologically either the diphtheritic character of the lesions or the presence of the bacillus in the milk. Although these facts were pointed out many years ago, only two observations have been published claiming to have demonstrated the presence of diphtheria bacilli in the lesions of cows. One was the interesting outbreak investigated by Dean and Todd (1902). The milk of two cows was used chiefly by the family of the owner and his servants and the surplus was sold to a few individuals. Two of these persons suffered from typical diphtheria and several had sore throats. The cows were found to be suffering from a disease, which had commenced about 10 days before, characterised by the following lesions. On the udders and teats of both papules and ulcers covered with dark brown scabs were present. The papules were, on the average, the size of a pea, and had a markedly indurated base which extended into the subcutaneous tissue. No vesicles were seen at this examination. The majority of the lesions were in the form of ulcers covered with dry brown crusts and varied from 2 to 2·5 cms. in diameter. On removing the crust from one of these ulcers there was exposed a slightly moist, fairly smooth surface, with an elevated puckered cicatricial-looking margin. The largest lesion at this stage on the udder measured 2 × 1 inches.

In cow 1 there was no evidence of mammitis, there was an abundant secretion of apparently normal milk, and the general health appeared to be little, if at all, affected. In cow 2 there was a distinct mammitis affecting a posterior quarter of the udder. In this case the milk was scanty, ropy, and semi-purulent looking. Cultures, taken from the lesions and from the milk of both cows, showed typical virulent diphtheria bacilli, whose effects on guinea-pigs could be neutralised by antitoxin. The toxigenic power of bacilli from three sources, namely, from the lesions and milk of cow 1 and the throat of a patient, was tested, and was found to be the same.

As controls cultures made from the teats of 13 apparently healthy cows in a small dairy farm showed no diphtheria bacilli.

With a view of further investigating the condition a healthy cow (No. 3) was milked immediately after the diseased ones by the same attendant. This animal developed several vesicles on the teats, but diphtheria bacilli could not be grown from the contents. Crops of papules continued to develop for a fortnight.

Two calves were shaved and scarified on the abdomen and inoculated with crusts from the experimentally infected cow No. 3. These animals developed papules, followed by shallow ulcers covered with brown crusts, but diphtheria bacilli could not be found in the lesions.

From these experiments the authors came to the conclusion that the lesions in the cows were not due to the diphtheria bacillus, but that the latter had probably been superadded to them in some way and multiplied in them.

The second example was investigated by Ashby (1906). By its distribution he became convinced that a diphtheria epidemic, which occurred between July 31st and August 26th, 1904, in the villages of Twyford and Ruscombe, containing about 2250 persons, was spread by infected milk. In all 75 persons living in 43 houses were attacked, of whom 64% were over 15 years of age. These cases were proved by bacteriological examination. Milk was supplied to the two villages by three dairymen, who may be designated X, Y, and Z. One of the houses invaded by diphtheria was supplied by X, 15 of them were supplied by Y alone, 17 were supplied by Z alone, and 10 were supplied by both Y and Z. The cows belonging to Y and Z were inspected on August 20th. It was found that all of the teats of two of Z's cows were badly ulcerated, and the teats of three other cows were affected to a lesser degree. Cultures taken from the ulcers on the teats of the worst two cases were examined at the Lister Institute of Preventive Medicine by Dr Alfred MacConkey, who isolated a diphtheria bacillus, fully virulent for guinea-pigs. Control guinea-pigs could be protected with antitoxin. From this organism a toxin was prepared of which $\frac{1}{40}$ c.c. killed a 245 grm. guinea-pig in 48 hours. Dr G. Dean also examined cultures independently, and proved the organism to be a true virulent diphtheria bacillus. Cultures from a sample of milk showed only non-pathogenic diphtheria-like bacilli.

"Whether the eruptive disease of the teat was a specific diphtheritic infection of the cow, or whether there was a specific contagious eruptive condition apart from diphtheritic infection cannot now be told; but it is certain that the diphtheria bacillus was present in the pathological lesions of the cow."

This report is accompanied by several excellent photographs of the teats of the affected cows as well as tables giving the results of the inoculation experiments on guinea-pigs.

Summary of Observation on Diphtheria in Cows.

Klein's experiments lead to the conclusion that cows can be experimentally infected with diphtheria, and that as a result of the infection certain lesions may be produced on the teats and udders which contain diphtheria bacilli, and that diphtheria bacilli may be present in the milk apart from these lesions. The experiments of Abbott and of Ritter do not, however, confirm Klein's observations, and most of those who have criticised these experiments hold that there is no evidence that diphtheria is a bovine disease. Further experiments are therefore needed before a definite conclusion can be arrived at.

Several authors have pointed out that in epidemics of diphtheria, in which the milk has been strongly suspected as the agent by means of which the disease has been transmitted, some of the cows supplying the milk have been found to be suffering from lesions of the udder and teats. In only two cases, those investigated by Dean and Todd (1902), and Ashby (1906), has any bacteriological evidence of the infection of the milk by the cows been produced. In the former case, although the milk and the lesions on the teats contained virulent diphtheria bacilli, further experiments led to the conclusion that the diphtheria bacilli were not the causal agents of the bovine disease, but had been in some way superadded. In the latter case virulent diphtheria bacilli were found in the lesions on the teats, but neither their origin nor their relation to the ulcers was determined. On this subject also further careful investigations are needed[1].

VII. Diphtheria in the Horse.

The experimental results of the injection of living bacilli or toxins into horses are dealt with later (Section V). The only instance of a horse suffering from diphtheria and communicating the disease to man has been published by Cobbett (25. VIII. 1900). A little girl having fallen ill of diphtheria, Dr Fraser, the Medical Officer of Health of Portsmouth, while seeking the source of infection, found that a pony belonging to the child's father was ill with a purulent and slightly sanguineous discharge from the nose. Subsequently the animal suffered from enlargement of the glands under the tongue and tracheal obstruction, with difficulty of breathing and retraction of the abdominal wall. The bacillus isolated from the discharge was morphologically a short diphtheria bacillus, and on careful investigation was found to behave in all ways like the diphtheria bacillus. It formed a powerful toxin, and the effects of the injection of both living bacilli and of toxin were neutralised by antitoxin.

---

[1] For milk epidemics and diphtheria-like bacilli in milk see Index.

Cobbett considered this observation of considerable practical importance, since it proves that the horse is liable to nasal and laryngeal diphtheria, and points out a hitherto unsuspected channel by means of which the infection can be carried to man, and of scientific interest because it has a direct bearing on the question of the origin of antitoxin in untreated animals. He concludes by remarking that "the fact that diphtheria antitoxin is present in many horses in this country and on the Continents of Europe and America suggests that diphtheria is a common disease among these animals....It is therefore possible that the horse may be found to play a not inconsiderable part in the transmission of diphtheria."

A leading article in the *Journal of Comparative Pathology and Therapeutics* (29. IX. 1900, p. 248), while admitting that the above instance is one of equine diphtheria, severely criticises Cobbett's deductions as follows: "These, we venture to say, are very rash suggestions. Dr Cobbett is apparently under the impression that equine pathology is an unexplored field and that Veterinary Surgeons have been treating cases of equine diphtheria for generations without ever suspecting the nature of the complaint. In that he is certainly mistaken. The bacteriology of nasal discharges in the horse is probably as well known at this present moment as that of like conditions in man, and it is beyond doubt that diphtheria of the horse is an exceedingly rare condition. That it is maintained by transmission from animal to animal of the equine species is incredible, and it will probably be a long time before a second case of equine diphtheria is met with."

VIII. THE PRESENCE OF DIPHTHERIA BACILLI IN THE BLOOD AND ORGANS OF EXPERIMENTAL ANIMALS.

While many of the earlier investigators were unable to detect any bacilli in the blood and organs, except in the neighbouring lymph glands, of animals dead from experimental diphtheria, later observers have been able to demonstrate their presence in some cases.

The bacilli can be detected microscopically, and also cultivated from the site of inoculation, in most animals unless death has been delayed for some days. The neighbouring lymph glands are also generally infected.

Roux and Yersin (1888) inoculated a series of guinea-pigs subcutaneously and killed specimens every two hours. After four hours oedema was found at the site of inoculation. The bacilli increased in numbers up to eight hours, but after this time their numbers decreased. Except in one instance they were unable to find bacilli in the organs or pleural effusion. Métin (1898) observed a decrease in the numbers of bacilli two hours after inoculation, and Park (1900) says that the bacilli multiply but little in the tissues.

The largest number of experiments on this subject has been made by Wright (x. 1894). He examined the organs of guinea-pigs with the following results:

Organ	Number examined	Number of times diphtheria bacilli found	Percentage
Liver	155	19	12·2 °/₀
Spleen	152	15	9·8 °/₀
Heart's Blood	153	7	4·5 °/₀
Kidney	151	4	2·6 °/₀

Zarniko (1889) first observed the bacilli in the necrotic foci of the liver, and Abbott and Ghriskey (1893) in lesions of the omentum. Not infrequently the bacilli have been seen in sections and preparations from the organs, but cultures have remained sterile. Abbott (1894), for example, observed bacilli in the subcutaneous lesions of experimental cows, but was unable to cultivate them, and Flexner and Anderson (1898, Expt. H), although they saw bacilli in smears from the lungs, found that in some cases cultures remained sterile.

The latter observers remark "that even after considerable numbers of bacilli in pure cultures have been inserted through the trachea into the lung, their recovery from these situations was often attended with much difficulty and sometimes was impossible."

Métin (1898) found that in the case of rabbits, inoculated with pure cultures, the bacilli seemed to multiply in the organs after death. Cultures from the spleen immediately after death often remained sterile, but the bacilli could be obtained from it after incubation for 48 hours. He also endeavoured to ascertain when the bacilli disappeared from the blood after intravenous inoculation, by examining samples of blood drawn at intervals from the ear vein. A few colonies were obtained by culture half an hour after injection, but not four hours after. The animal was killed in six hours, but no bacilli could be found in the spleen before or after incubation. A second experiment gave the same results in regard to the presence of bacilli in the blood. This rabbit was killed 30 hours after injection. Although the organs and blood yielded no bacilli in cultures made immediately after death, colonies were obtained from the spleen after 48 hours' incubation. Animals killed 48 and 68 hours after inoculation gave the same results. A series of experiments on guinea-pigs subcutaneously inoculated gave comparable results in regard to the rapid diminution of the bacilli at the site of inoculation and their presence in the spleen. Experiments, in which mixed cultures of diphtheria bacilli and cocci were introduced into the circulation of rabbits and under the skin of guinea-pigs, showed that the bacilli and cocci could be detected in the blood of the rabbits and in the subcutaneous oedema of guinea-pigs for many hours. Cultures from the organs and blood immediately after death also showed both organisms. Métin therefore thought that under these conditions the diphtheria bacilli were able to multiply in these situations during life.

IX. DIPHTHERIA-LIKE ORGANISMS FOUND IN ANIMALS[1].

Rats and mice.

Klein (1903) obtained from the hepatised lung of a white rat a bacillus closely resembling the diphtheria bacillus in its morphology, behaviour towards Neisser's stain, and cultural characters, which he has called *Bacterium muris*. It forms acid in glucose broth and is pathogenic to rats and guinea-pigs. In the case of an inoculated rat the whole of one lung became hepatised, and the other contained gray patches, and both showed ecchymoses. The liver and spleen were hyperaemic and the glands injected. In smears from the lungs large and small forms of the bacilli showing metachromatic staining were plentiful. Pure cultures were obtained from the lung and heart's blood. Another rat dying in 18 days showed similar changes. In guinea-pigs a hard tumour is formed at the site of injection which reaches a considerable size, and which gives rise to an abscess containing thick pus, in which the bacilli can be demonstrated. In all cases the animal recovers. Antitoxin has no effect.

Bergey (1904) found organisms, morphologically resembling diphtheria bacilli, in abscesses occurring spontaneously in laboratory mice, and Dean (1905, p. 103), while investigating a leprosy-like disease in rats, succeeded in obtaining from two of the affected animals cultivations of a diphtheroid bacillus. In young cultures on agar or serum "it has the form of a small diplococcus, or is sometimes very like Hofmann's bacillus. On the second or third day it becomes longer and has all the appearances of a typical diphtheria bacillus

---

[1] For an account of the diphtheroid bacilli found in milk see Chapter VIII.

with segmentation, club-shaped ends, etc., and later it may form short much segmented and branching filaments." On serum the colonies are indistinguishable from those of the diphtheria bacillus. On agar the growth is slower and the colonies more delicate. On broth it forms no surface film, but grows in the form of clumps along the test-tube wall, or as a flocculent deposit at the bottom. Glucose broth rapidly becomes acid. It forms no visible growth on potato. "This diphtheroid bacillus has a feeble pathogenic action on young rats. Three out of four young rats that received 2 c.c. of a broth culture a week old died within a week. Old rats proved resistant."

Horses.

Paffenholz (1895) found diphtheria-like bacilli in a tumour of the mamma in a mare.

Dogs and guinea-pigs.

As the xerosis bacillus appears to be a common inhabitant of the normal human conjunctiva, the writer (Graham-Smith, 1904, p. 307) made cultures from the eyes of a few animals to ascertain whether similar organisms are to be found there.

The eyes of three dogs, three rabbits, and 17 guinea-pigs were examined, and organisms closely resembling the xerosis bacillus were found in the eyes of the dogs and guinea-pigs, but not in the eyes of the rabbits.

Bacillus xerosis canis (Pl. XVI, fig. 14).

Origin. From the conjunctival sacs of the three dogs examined.

On serum the colonies only make their appearance after 2—3 days' growth, though in later subcultures the growth is a little more rapid. After 4—5 days' growth the colonies are of large size. Except for their size the colonies are indistinguishable in appearance from those of the diphtheria bacillus, but tend to adhere to the medium. The organisms are long, curved, and stain well, showing well differentiated, short, dark segments, separated by narrow light bands crossing the bacillus transversely. Clubbing in some specimens is well marked. These organisms resemble closely the pseudo-diphtheria type of Hofmann's bacillus (Pl. XV, fig. 7). By Neisser's method variously shaped polar bodies are seen in large numbers in each bacillus. Some are large and round, others elongated transversely across the bacillus, and many are exceedingly small. The organisms are non-motile, and stain well by Gram's method. On agar after 2—3 days' growth small grayish colonies with irregular edges and darker centres appear. On agar stab cultures the surface growth is small and almost transparent, but in the depth a few medium-sized round colonies develop. On gelatin no growth was obtained. No visible growth occurs in potato.

Broth after 48 hours remains clear, but small granules are found at the bottom of the tube, which when shaken up float in lines as if held in position by invisible threads. The growth in glucose broth is similar, and its reaction after 48 hours is neutral. It is non-pathogenic to guinea-pigs.

This organism differs only slightly from the diphtheria bacillus in morphology, but differs from it in its growth on serum, gelatin and broth, and its reaction in glucose broth. It resembles closely in many respects the xerosis bacillus from the human eye.

Fourteen (82%) out of 17 guinea-pigs' eyes examined showed similar organisms. They resemble the organism just described in morphology, staining characteristics, and in their growth on all media except serum. On serum their colonies are the same in most cultures, but in some cases larger colonies develop which have a raised centre and raised rim.

These observations indicate that organisms closely resembling the diphtheria bacillus in morphology, and in many respects in cultural peculiarities, but totally unconnected with them, are common inhabitants of the conjunctival sacs of some animals.

X. THE LESIONS OF EXPERIMENTAL DIPHTHERIA IN BIRDS.

Fowls, pigeons, and other birds may be killed by the inoculation of diphtheria bacilli, but except in the case of tracheal inoculations do not show very characteristic lesions.

Subcutaneous and intramuscular injections of doses of 1 c.c. of virulent broth cultures kill pigeons in 60 hours. In the former case a small layer of gelatinous oedema is observed and a general congestion of the internal organs without special lesions. Intramuscular injections produce the same general conditions, and the muscle is swollen, soft, yellowish in colour, and easily broken down. Bacilli can be isolated from the broken-down muscular tissue, and histologically the organisms may sometimes be seen penetrating the fibres. There is no marked congestion of the suprarenal glands.

Tracheal inoculations.

The majority of observers, who have made experiments on this subject, state that more or less extensive pseudo-membranes may at times be produced by inoculating the abraded mucous membrane of the trachea in birds. These lesions cannot, however, be produced with certainty. According to Henke (1898) only 25% of the inoculated birds exhibit membrane formation. While some observers have confirmed this observation others seem to have been unable to infect fowls in this manner. Harrison (1902), for example, could not produce any effects in fowls either by subcutaneous or submucous inoculations, or by rubbing cultures on to the abraded surface of the trachea.

XI. THE RELATIONSHIP OF AVIAN TO HUMAN DIPHTHERIA.

Diphtheria of fowls and pigeons has been made the subject of numerous investigations; and when we examine the literature, we are immediately struck with the differences of opinion regarding the disease. On one side we have those who believe that the one disease in man and in birds is identical; and, on the other side, those who believe that the one disease has no relation to the other.

The various writers and investigators on this subject may be conveniently grouped under two heads:

(1) Those who have investigated the disease as it occurs in fowls and pigeons, by the usual methods employed in working out infectious diseases.

(2) Those who have made observations without experimental research, and who did not employ bacteriological methods to support or controvert their views, either for or against the identity of the disease as it appears in birds and man.

(i) *Experimental Investigations on Avian Diphtheria.*

(*a*) *Observations in which the disease was found to be due to organisms bearing no resemblance to the diphtheria bacillus.*

Only a few of the more prominent investigations are quoted in which diphtheria-like organisms have not been discovered.

(1) *Bacilli.* Loeffler (1884, p. 482), isolated from the pseudomembranes in the mouths of pigeons, which died from an infectious form of diphtheria prevalent in Germany, a bacillus, which, when

inoculated in pure culture, reproduced the disease. It was non-pathogenic to guinea-pigs, rats and dogs. Loeffler's observations were soon afterwards confirmed by Pütz (1887), Cornil and Babes (1890), Méguin (1891) and Ménard (1890).

Moore (1895) isolated a bacillus resembling that causing swine plague. Loir and Ducloux (1894, p. 599) studied in Tunis an outbreak which affected fowls, ducks, sparrows, pigeons and turkeys. From the tissues and fluids of all these cases they isolated a motile, non-liquefying bacillus with rounded ends which gave yellow colonies on potato. It did not stain by Gram's method, and was pathogenic for fowls, pigeons, ducks, sparrows and rabbits, but not for guinea-pigs. Cases of so-called diphtheria were common amongst those who attended the birds, and from one of these the same bacillus was isolated. The inoculation of the bacillus into healthy fowls gave them the disease.

Nocard and Leclainche (1903, *Les Maladies microbiennes des Animaux*, 3rd edition) state that the bacillus of avian diphtheria is analogous in its form to that of the haemorrhagic septicaemias.

Guérin (1903) has recently investigated several outbreaks of fowl diphtheria and isolated a cocco-bacillus which in pure cultures reproduces the disease in fowls. By successive inoculations on the conjunctiva of pigeons its virulence can be so raised that it produces death in fowls in 24 hours. He further succeeded in immunising fowls against the disease by means of a specific anti-serum. In consequence of his researches he came to the conclusion that human diphtheria is entirely distinct from that of the fowl. During three years this author never saw or heard of a case of human infection.

(2) *Cocci.* Cocci have been found in the false membranes by numerous observers.

(3) *Protozoa.* Pfeiffer (1889, p. 363), Babes and Puscariu (1890, p. 376), and Piana and Galli-Valerio (1894, *Moderno Zooiatro*, quoted by Harrison), as well as other later observers, have found flagellata and other protozoa in the throats of affected birds. In some cases they have been associated with Loeffler's bacillus of avian diphtheria.

Harrison (1902) has given the subject of avian diphtheria great attention, his work extending over a period of four years, and involving observations and inoculations on 300 fowls. He never met with the diphtheria bacillus in cultures from over 200 fowls, which had died from avian diphtheria or had been examined at various stages of the disease. His observations on the structure of the false membranes of fowls show that they are composed almost entirely of pus, some granular masses, and débris of epithelial cells, and therefore differ entirely in their histological structure from the pseudo-membranes of true human diphtheria. He isolated specific bacteria from the diseased birds, and reproduced the typical disease in healthy birds by injection. The virulence of the organisms could be exalted by successive passages through pigeons. He also isolated from birds suffering from roup the *Bacillus pyocyaneus*, and reproduced the disease by its inoculation. Finally his experiments with the diphtheria bacillus convinced him that it was not pathogenic for hens.

(*b*) *Observations in which diphtheria-like organisms have been discovered and looked upon as the causal agents.*

De Verey (1895) stated that he found diphtheria bacilli in two cocks suffering from avian diphtheria, and regarded the bacilli as the cause of the disease, and Fagnet (1898) also asserted that he had found typical virulent diphtheria bacilli in one type of avian diphtheria. Gallez (1898) discovered in the nasal mucus of fowls, affected with contagious catarrh, a bacillus identical with the diphtheria bacillus in staining reactions and in cultural characters. Its virulence for guinea-pigs was very slight, though these animals could be killed with very large doses. Cultures inoculated into fowls reproduced the typical catarrh. Ferré (1898) describes a bacillus isolated from diphtheritic birds, which retains Gram's stain, and occurs in long and short banded forms. False membranes are produced in rabbits, pigeons and fowls by the inoculation of cultures, and paralyses in guinea-pigs and fowls by the injection of its toxins. He seems to have found the same organism in the throats and cloacae of healthy fowls. Gratia and Lienaux (1898) isolated from pigeons suffering from a diphtheritic disease an organism resembling the diphtheria bacillus in most of its characters. It was segmented, retained Gram's stain, showed polar granules, and varied greatly in length. In culture it grew well on serum, and produced a granular deposit and acid reaction in broth. No toxin was however produced. Gordon Sharp (1900) found a diphtheria-like bacillus in fowls suffering from roup, and Turner (1900) made similar observations. Neither of these authors seems to have tested the pathogenic properties of their bacilli.

Stevenson (1898), apparently on the results of experiments on diseased fowls with diphtheria antitoxin, stated that fowl diphtheria or "roup" was caused by the diphtheria bacillus and was identical with the human disease. Harrison (1902), however, showed that in his cases neither anti-diphtheritic, nor normal horse, serum had any effect on the course of the disease. All the authors cited above consider that in some outbreaks at least avian diphtheria is caused by the true human diphtheria bacillus, though the organism is frequently of low virulence.

(c) *Observations in which diphtheria-like organisms have been discovered in diseased or healthy birds, but were not regarded either as the causal agents or as true diphtheria bacilli.*

McFadyean and Hewlett (1900) isolated and cultivated bacilli, morphologically resembling diphtheria bacilli, both from healthy pigeons and others suffering from pigeon "canker." They describe these organisms as resembling diphtheria bacilli in size and parallel arrangement. They retain Gram's stain, and show polar bodies by Neisser's method. Growth on serum varies, some examples producing dry and abundant growth like the xerosis bacillus, and others moist colonies like the diphtheria bacillus. These organisms produce acid and indol in broth, and are entirely non-pathogenic to mice and guinea-pigs, and in culture have no effect on pigeons. Harrison (1901) found the same organism in the throats of healthy pigeons. Guérin (1903) in 78 cultures from the throats of fowls twice isolated a bacillus morphologically resembling the diphtheria bacillus, but which produced no toxin, and he also states that Malvoz had once met with this organism. Streit (1904) also found similar bacilli which did not retain Gram's stain. The writer (Graham-Smith, 1904, p. 314) also found an organism resembling the diphtheria bacillus in the throat of a healthy fowl, and suggested the name *Bacillus diphtheroides gallinarum*. Its morphological and cultural characters are as follows:

Bacillus diphtheroides gallinarum (Pl. XVI, fig. 5).

Origin. From the throat of a fowl. There was a hard tumour on the side of the left mandible, but the bird was otherwise normal.

On *serum* the colonies after 24 hours' growth resemble those of the diphtheria bacillus. Later the margins become crenated. In the first cultures the organisms were long, curved, and clubbed, with three or four well-marked polar bodies. Slight swellings were present round the polar bodies. The rest of the protoplasm stained lightly, but slight signs of segmentation were present. They resembled closely the diphtheria bacillus shown on Pl. VI, fig. 1. In subcultures well-marked segments are seen. These organisms are non-motile, and retain the stain deeply by Gram's method. On *agar* small, filmy, transparent, gray colonies are formed. The organisms are very long, thick, curved, clubbed, and well segmented, but no polar bodies are present. On agar stab cultures an almost transparent film is formed on the surface, and minute round colonies along the needle track. On *gelatin* after five days' growth minute, round, almost transparent colonies appear. On gelatin stab cultures there is very little surface growth, and a very scanty growth of minute colonies along the needle track. No visible growth occurs on *potato*. *Broth* remains clear and there is a slight granular deposit. *Glucose broth* shows a neutral, or slightly alkaline reaction. *Indol* is produced. Non-pathogenic to guinea-pigs.

As will be seen from the above description this organism very closely resembles the diphtheria bacillus except in its lack of virulence to guinea-pigs and in the production of an alkaline reaction in glucose broth.

Turkeys.

In studying an infectious disease of turkeys, characterised by gelatinous infiltration of the infra-orbital dilatation of the nasopharyngeal system of air-sacs, the writer (Graham-Smith, 1907) has encountered two species of diphtheroid bacilli, neither of which seems to bear any relation to the disease. The species which more closely resembles the diphtheria bacillus was found in groups usually composed of three to ten individuals, but occasionally containing as many as 40—50, in smear preparations of the gelatinous material. Most of the organisms were curved, showed well-marked segmentation, and well-defined polar bodies. In culture this species has the following characteristics.

On *serum* after 24 hours' growth at 37° C. the colonies are small, round, yellowish and dry-looking. After three days' incubation the colonies become very large. The centre is much raised and deep yellow in colour and is surrounded by a flat pale yellow zone. The whole colony has a very granular appearance. If the colonies are crowded together they coalesce to some extent to form a film which appears to have a wrinkled surface owing to the irregularities in the height of the component colonies. The bacilli bear a remarkable resemblance to true diphtheria bacilli. They are of medium length, with rounded ends, and are slightly curved. Clubbed extremities are common and many have irregularities in their length. Some branched forms were seen. They retain Gram's stain, show polar bodies, both terminal and central, with Neisser's stain, and differential staining of the protoplasm with methylene blue. In some dark and light bands alternate causing a segmented appearance, whilst in others these areas are irregularly placed. They are non-motile, do not form spores, and have no characteristic arrangement. On *agar* large white granular, irregular, dry-looking, heaped up colonies are formed, which after a few weeks' growth increase in size and become wrinkled. The bacilli are shorter than when grown on serum and many coccus-like forms can be found after 48 hours' growth. On *potato* large, yellow, dry-looking, discrete colonies are produced. At first the bacilli are like those from the serum cultures, but later large irregular involution forms are common. Good growth occurs on *gelatin*, and large dull-yellow, granular colonies are formed. The medium is not liquefied. In *broth* a whitish wrinkled film is produced on the surface, and a yellowish flocculent deposit. Acid is produced in media containing *glucose*, *galactose*, and *laevulose*, but not in media containing *lactose* or *glycerine*. The organism is non-pathogenic to guinea-pigs.

(ii) *Opinions as to the Relationship of Human and Avian Diphtheria based on Clinical Observations.*

Clinical observations, however trustworthy, of the transference of a disease characterised by the formation of false membranes from birds to man or from man to birds, so long as they are unsupported by bacteriological proof, cannot be taken as evidence of the transmission of true diphtheria. Loir and Ducloux's (1894) observations clearly demonstrate this point. On this account, although there are many instances quoted in the literature of such outbreaks, only a few of the more noteworthy examples are cited.

Gerhardt (1883) published the report of an outbreak of diphtheria amongst two-thirds of the employees of a poultry establishment at Nesselhausen, where thousands of fowls had succumbed to diphtheria. One of the employees, pecked by a diphtheritic rooster on the hand and foot, subsequently showed false membranes in these situations. No cases of diphtheria were present in the environs of Nesselhausen at the time.

According to Paulinis (1888) the Greek island of Skiatos, in which diphtheria had never previously been known, was infected after the introduction of diphtheritic turkeys. Barbier (1899, p. 37) frequently saw diphtheria amongst fowls which lived beside isolated buildings for diphtheria patients, and records the case of a woman, 67 years of age, who was attacked by diphtheria after supervising the disinfection of a poultry building in which fowls suffering from diphtheria had been kept. No other case existed in the neighbourhood, and she had never been out of the house for the previous three weeks.

Debrie (1892) reported an interesting case. Some soldiers suffering from diphtheria were admitted to the hospital at Sebdou. Shortly afterwards the fowls, which were looked after by a hospital attendant, showed symptoms of diphtheria.

On the other hand numerous observers have stated that they have never seen cases of human diphtheria amongst those who were in charge of diphtheritic birds.

Conclusions.

Those who have adopted experimental methods in investigating avian diphtheria have shown that the great majority of epidemics have been due to organisms totally distinct from the diphtheria bacillus. In a few instances only have diphtheria-like organisms been found, and most of these have been completely devoid of virulence for guinea-pigs. Moreover the results of experimental inoculation of the trachea in birds with diphtheria bacilli show that they are frequently resistant to this organism, and therefore unlikely to acquire the disease in epidemic form. The clinical observations, which support the identity of the two diseases, are not very convincing, owing to the lack of corroborative bacteriological evidence.

On the whole therefore these investigations point to the conclusion that true diphtheria, produced by the diphtheria bacillus, if it ever occurs, is a very rare disease in birds.

Friedberger and Fröhner (1904, p. 221) in their well-known work on veterinary pathology summarise the whole question of animal diphtheria in the following words:—"The diphtheritic diseases of domesticated animals are in no way related to human diphtheria. No indisputable case of the transmission of diphtheria to man from an animal has yet been proved. The cases recorded in the medical literature, of the alleged transmission of infection, especially those of chicken-diphtheritis supposed to have been conveyed to man, are, on closer examination, reduced to mere assumptions, the forming of which have been due to entire ignorance of veterinary pathology. Even the statement of Gerhardt—which is the only one of the kind worthy of being quoted—that in a chicken-hatching establishment two-thirds of all the workmen attending on the fowls which were suffering from diphtheritis, became affected with pharyngeal diphtheria, cannot be substantiated; for Gerhardt did not observe the case personally. Considering the wide distribution of chicken diphtheritis, especially in hatching establishments, and presuming that this disease is identical with human diphtheria, a very large number of people would necessarily become infected daily, and reports of such cases would not be, as they are, extremely rare. We have examined thousands of domestic fowls and pigeons suffering from diphtheritis without having either seen or heard of a single case of infection. On the other hand, no indisputable proof has yet been given that any disease similar to human diphtheria has been transmitted, either experimentally or accidentally, to any of the domesticated animals."

Holmes (1904) in an article entitled "An Outbreak of Diphtheria associated with a similar Disease among Fowls and a Vesicular Eruption on the Udders of Cows," describes a very remarkable epidemic. Owing to its peculiar features it has not been included in the discussion on avian diphtheria, but it is recorded by itself. The outbreak occurred at Muktesar, a bacteriological station in the Himalayas, situated 23 and 16 miles respectively from the nearest European settlements, and 3 to 4 miles from the nearest native villages. The only inhabitants consisted of the staff and their families (four children). In June one child developed a nasal discharge, and a few days later another suffered from a disease which was diagnosed as diphtheria, and eventually died. Antitoxin was injected in this case, but somewhat late in the disease. There had been no case of diphtheria in the surrounding districts or stations for years, and no clue was obtained as to how the infection was brought. Two months previously a cow had been purchased which developed a cough and general illness, but recovered. Later a second cow was bought and placed near the other, and developed a similar illness. It was at this time that the child developed rhinitis and this circumstance led to a careful examination of the cows. It was then found that there were newly formed vesicles, ulcers, and pustules on the udder of the second cow, and healed lesions on that of the first cow. Cultures obtained from the vesicles showed bacilli somewhat resembling diphtheria bacilli, but non-pathogenic to rabbits and fowls. No further investigation of these organisms seems to have been made.

A month before the children's illness began a disease broke out amongst the fowls, which continued intermittently for several months, each outbreak following a turning up of the subsoil to a depth of 2 to 6 feet. From the soil on many occasions a bacillus was obtained identical in morphology with those derived from the birds, but non-virulent to rabbits.

Holmes carried out a large series of inoculation experiments with cultures and other material derived from the diseased children and fowls, which are summarised below:

A. EXPERIMENTS ON ANIMALS WITH CULTURES DERIVED FROM THE SICK CHILDREN.

I. *Fowls.* (1) A fowl inoculated on the abraded surface of the throat with a culture derived from the dead child died in seven days, with the larynx, trachea, pharynx, and nasal cavities covered with a fibrinous adherent membrane. No other lesions were discovered at the autopsy. Smears made from the membrane and heart's blood showed a bacillus with rounded ends and darkly stained polar granules. In cultures the organisms resembled diphtheria bacilli. (2) A fowl injected subcutaneously died in 2½ days. At the site of injection a white fibrinous deposit was found between the muscles, and the surrounding tissues were injected and oedematous. Smears and cultures showed the same organisms as were found in the first fowl. (3) Intramuscular injection gave the same results.

II. *Bulls.* (1) A bull subcutaneously inoculated behind the shoulder with 5 c.c. of a 48 hours' broth culture developed very extensive oedema, extending down the leg, and died in 60 hours. At the autopsy an extensive yellow gelatinous oedema was found under the skin, and layers of white coloured fibrinous material between the muscles. The neighbouring glands were enlarged, congested, and oedematous, but no other lesions were found. A second bull inoculated in the same way died in 60 hours.

Six other bulls were also injected with minute doses.

Bull No. 3 received 1 loopful of broth culture and died in 5½ days.
,, 4 ,, 1 ,, ,, ,, ,, 6 ,,
,, 5 ,, 1 ,, ,, ,, ,, 6 ,, (culture 1 month old).
,, 6 ,, 2 ,, ,, ,, ,, 3 ,,
,, 7 ,, 2 ,, ,, ,, ,, 2 ,, (culture 6 weeks old).
,, 8 ,, 2 ,, ,, ,, ,, 64 hours ,, ,, ,,

In the oedema and heart's blood in all cases bacilli with bipolar staining, like those described in the fowls, were found, and in all those instances in which cultures were made from the heart's blood the organisms were isolated.

III. *Rabbits.* The pathogenic properties of cultures of these organisms towards rabbits were tested on several animals. All the rabbits died except one which had received a dose of antitoxin, and the bacilli were cultivated from the oedema and heart's blood.

	Method of inoculation	Dose	Result
Rabbit 1.	On abraded conjunctiva	?	Died in 4 days with fibrinous exudation on the conjunctiva.
,, 2.	On abraded surface of throat	?	Died in 36 hours with pseudo-membrane in trachea.
,, 3.	Subcutaneous	1 loopful	Died in 2 days. Yellow gelatinous oedema and injection at site of inoculation. Suprarenals reddish.
,, 4.	Subcutaneous	1 ,,	Died in 48 hours.
,, 5.	Subcutaneous	1 ,,	Died in 36 hours. Yellow gelatinous oedema and injection at site of inoculation. Suprarenals reddish.
,, 6.	On abraded surface of throat	?	Died in 5 days.
,, 7.	Culture applied to abraded throat the day after the injection of ·5 c.c. of diphtheria antitoxin	?	Remained well.

IV. A *cat* inoculated on the abraded surface of the throat remained well. Three weeks later it received a subcutaneous inoculation of one loopful and died in four days. A white fibrinous deposit was found at the site of inoculation, surrounded by yellow oedema. Cultures were obtained from the blood, organs, and oedema.

V. A *dog* subcutaneously inoculated with half a loopful of culture died in three days. The subcutaneous tissue was deeply injected, and round the seat of inoculation there was a large amount of oedema.

VI. Two *rats* were inoculated subcutaneously each with half a loopful of culture, one died on the 3rd day and one on the 4th. There was oedema at the site of inoculation, and the organisms, recovered from the heart's blood, were fatal to rabbits.

The Natural Disease in Fowls.

A *fowl* (A) naturally infected showed fibrinous adherent membrane in the pharynx, larynx, trachea, and nasal cavities, but no other lesions. In smears from the membrane and heart's blood bipolar staining organisms, like those previously described, were found and isolated by culture.

Thirteen fowls and four ducks all showed more or less extensive lesions of this nature, and in two cases the oviducts of two diseased hens showed similar membranes. The bacilli seen in smears made from these membranes are described as being bipolar with rounded ends analogous to those of haemorrhagic septicaemia, non-motile, and not retaining the stain by Gram's method. In cultures they varied in form, often greatly resembling diphtheria bacilli. "In a blood-serum culture 24 hours old a thin transparent coating is visible, at the borders of which were isolated colonies of grayish colour and margin finely indented."

B. EXPERIMENTS ON ANIMALS WITH CULTURES AND MATERIAL DERIVED FROM DISEASED FOWLS.

I. *Fowls.* Several healthy fowls were inoculated with material from the false membrane by scraping the throat, and also subcutaneously with blood taken from the hearts of fowls which had died of avian diphtheria. Also many fowls and pigeons were inoculated with cultures from the blood and pseudo-membranes of diseased fowls, but except in one instance with no result. In order to raise the virulence of the contagion the method practised by Guérin was tried, namely, inoculating pigeons in the conjunctiva with some of the false membrane taken from an affected fowl. This also failed to have any effect. The one bird which was infected developed the same lesions as the birds which acquired the disease naturally.

II. *Bulls.* (1) A bull, which received subcutaneously five loops of a 48 hours' agar culture obtained from a diseased fowl (A), died in four days, with very marked oedema. The organisms were found in the oedema and heart's blood. (2) A second bull, which received 1 c.c. of oedematous fluid obtained from bull No. 1, died in four days. The autopsy showed extensive oedema, 1000 c.c. of yellow fluid in the pleura and 1500 c.c. in the peritoneum. The apex of the heart was covered with gelatinous oedema.

III. *Rabbits.* (1) A subcutaneous injection of 2 c.c. of broth emulsion of the pseudo-membrane of a diseased fowl (A) killed a rabbit in 48 hours with only slight oedema. From the oedema and blood cultures were obtained. Subcutaneous injections of one loopful of a culture obtained from the blood of this rabbit had no effect on two fowls and a pigeon. (2) A rabbit, which received one loopful of a 48 hours' agar culture derived from rabbit No. 1, died in 48 hours without well-marked oedema. Pure cultures were obtained from the blood. (3) A rabbit inoculated subcutaneously with ·25 c.c. of oedematous fluid from bull No. 2 died in 12 days. The autopsy showed extensive oedema and 200 c.c. of yellow fluid in the peritoneum. Cultures were obtained from the fluid, oedema, and blood. (4) A rabbit which received ·5 c.c. of diphtheria antitoxin, followed next day by a loopful of a serum culture from rabbit No. 1, died in 15 hours, with lesions similar to those found in rabbit No. 1.

IV. *Cats, Dogs, and Rats* proved resistant to subcutaneous inoculation with emulsions of pseudo-membranes and cultures from various sources.

Holmes apparently considers that this is an instance of a true diphtheria outbreak occurring in children, cows, and fowls simultaneously, but the organisms obtained from all sources do not agree with diphtheria bacilli in their pathogenic properties, and they seem to differ to some extent in their morphology and cultural characters. Although a rabbit was protected by antitoxin from the bacilli obtained from one of the children, yet the septicaemic condition produced by the inoculation of minute doses into fowls, rabbits, bulls, cats, dogs, and rats differs entirely from the lesions produced in these animals by the injection of true diphtheria bacilli. Rats indeed are well known to be extraordinarily resistant even to enormous doses of virulent diphtheria bacilli. The bacilli derived from the infected birds, although producing a similar condition in bulls and rabbits, were almost non-virulent for fowls, and totally without effect on pigeons, cats, dogs, and rats. Moreover, a rabbit was not protected in any way by antitoxin.

Summary of Chapter VII.

The subcutaneous inoculation into a guinea-pig of a pure culture of a virulent diphtheria bacillus causes the death of the animal within three or four days with characteristic lesions, namely, a gray necrotic pseudo-membranous focus at the site of inoculation, more or less extensive gelatinous oedema, pleural effusion, and more or less well-marked congestion of the viscera, especially the suprarenal capsules. The bacilli are found in large numbers at the site of inoculation, and can sometimes also be recovered from the blood and organs. Intraperitoneal inoculations also cause the death of the animals. Pseudomembranes may be produced by inoculation of the mucous surfaces, and paralyses may be caused by the injection of dilute toxins, or living cultures and antitoxin. Rabbits, dogs, and cats are also susceptible. In the latter fatty degeneration of the cortex of the kidney is a marked feature of diphtheritic intoxication (Klein). Local lesions are produced in cows by the subcutaneous injections of cultures, and, according

to Klein, vesicular lesions also occur on the teats. The same observer thinks that the bacilli pass into the milk. These results have not been confirmed by other workers. Rats are very resistant to the diphtheria bacillus and its products. Birds (fowls, pigeons, etc.) can be killed by subcutaneous and intramuscular injections of diphtheria bacilli, and false membranes may occasionally be produced in the trachea by the application of cultures to the abraded surface.

The disease has been proved to occur naturally in the horse, and lesions associated with the presence of virulent diphtheria bacilli have been described in the cow. In each instance human subjects have been infected from these sources. Both cats and fowls have frequently been regarded as carriers of the disease, but the bacteriological evidence in support of these statements is unsatisfactory. Instances of natural infection amongst other animals are unknown.

Bacilli closely resembling diphtheria bacilli in many of their characters have been found in dogs, guinea-pigs, rats, fowls, turkeys and pigeons.

CHAPTER VIII.

THE MODES OF INFECTION IN MAN.

Transmission of the bacilli from patients, directly and through infected articles. Transmission of bacilli by persons suffering from atypical forms of diphtheria, Nasal catarrh, Fibrinous rhinitis, Tonsillitis, Otorrhoea. Transmission of diphtheria by healthy contacts. Mode of transmission from unrecognised cases and healthy contacts. Infection through infected articles, food substances, milk, animals, birds, air, dust, soil, drains and sewer-gas. Laboratory infection. Summary.

FOR some years before the recognition of the diphtheria bacillus it had been almost universally admitted that one of the most common modes of dissemination of diphtheria was from diseased persons to others. The discovery of the diphtheria bacillus led to a more accurate knowledge of the means by which the disease is spread. Numerous researches have made it certain that a considerable percentage of those who come into contact with the patients acquire the bacilli, but only suffer from the disease in a modified and clinically unrecognisable form, or remain healthy. Many of these persons probably owe their immunity to the presence of antitoxin in their blood (see p. 41). It is these persons who are the chief factors in the spread of the disease, since they are frequently neither isolated nor suspected, and in their turn distribute the infective agents to others. Statistics have already been given (pp. 181—188) showing the percentage of such infected healthy persons found amongst various classes of "contacts," and the methods by which the bacilli pass from one individual to another will only be discussed here.

(1) *The Direct Transmission of Bacilli from Patients to Healthy Individuals.*

Patients suffering from diphtheria frequently cough out with considerable force small pieces of membrane or masses of mucus or saliva containing bacilli, which may be deposited on the mucous surfaces or skin of those in attendance on them. Numerous instances of the transmission of the disease in this manner are given in the literature, but need not be quoted here. On several occasions also the attempt to clear the passages by applying the lips to a tracheotomy tube and removing the block by suction has resulted in the infection of the operator.

(2) *The Transmission of the Bacilli from Patients by Infected Articles.*

(*a*) It has already been shown that diphtheria bacilli may remain alive for a considerable length of time when enclosed in membranes or masses of expectorated mucus even after drying (p. 171). Park (1892) for example was able to demonstrate the presence of living diphtheria bacilli in "a bit of membrane no larger than a pin's head four months after its removal from the throat." Such fragments of membrane are obviously a source of continued danger. Linen and other articles soiled by the expectoration and discharges of diphtheria patients have also frequently conveyed the disease to other persons (Park and Beebe, 1895, p. 49).

Examples of such instances when occurring within a short time need scarcely be quoted. Although bacteriological evidence is lacking in some of them, several highly interesting cases have been cited by various observers showing the possibility of infection by soiled articles long after the date of their infection.

Carstairs (1901) recounts the case of a father and son who were cornet players being attacked by diphtheria. The instrument was put away. A younger member of the family having found the cornet played it and developed diphtheria in a week. There had been no case in the district for eight weeks previously. Bugbee (1904) states that certain library books, which had been used by a diphtheria patient the day before the appearance of the typical clinical signs of diphtheria (confirmed by cultures), were wrapped in brown paper and returned to the library. These were put in the cellar and fumigated. Eleven months later one of the committee found the parcel and opened it, and seven days later developed diphtheria. Warry (1895) reported an outbreak in which 23 (out of the 26) cases occurred amongst 43 adult females occupying a work room. He thinks that the speaking tubes, which were largely used, were instrumental in conveying the disease.

There is also some experimental evidence to show that diphtheria bacilli may remain alive on naturally soiled linen for a considerable time and even in dust and on hair, etc., although apparently not protected by dried secretions.

Trevelyan (1900) gives an instance in which diphtheria bacilli were cultivated from a handkerchief 11 weeks after it had been used by a child suffering from diphtheria. Wright and Emerson (1894) once cultivated virulent diphtheria bacilli from a brush used for sweeping a ward, and on three occasions isolated virulent bacilli from the dust clinging to the shoes of nurses. They also found a diphtheria bacillus of low virulence in one out of four cultures made from the hair of nurses, but numerous cultures made from the sweepings of the floor, scouring clothes, skirts of nurses and patients, finger nails, etc., were negative as regards the presence of the diphtheria bacillus.

Weichardt (1900) examined objects surrounding diphtheria patients by means of damp swabs, 50 samples being taken from the sick room and 250 from other parts of the house. Diphtheria bacilli were found three times, all from objects directly soiled by the patient, *i.e.*, a bottle, a neck cloth, and a spot on the carpet within ·5 metre of the patient's mouth. Hill (1902), by means of damp swabs, also found non-virulent diphtheria bacilli on two articles (toy and handkerchief) closely connected with the patients after the latter had been officially released from isolation following upon two consecutive negative bacteriological examinations. Fourteen doubtful, non-pathogenic bacilli were obtained from sheets, pillows, mattresses, bed frame and floor amongst the 331 other swabs examined. Three hospital wards, all occupied at the time of examination, were also investigated, using 197 swabs, with negative results. He mentions that the collector was careful to select exposed surfaces on sheets, pillows, furniture, walls and floor near the patients. At least two swabs, usually more, were rubbed on each area selected.

In order to test the efficiency of swabs for removing *B. diphtheriae* from wooden and cotton surfaces, on which bacilli had been dried, the following experiments were made. The wooden surface of an old varnished box and some new factory cotton were sprayed with the same emulsion of diphtheria bacilli made by suspending a 24 hours agar culture in water, and both were set drying in a protected place in diffuse daylight. The swabs were moistened in sterile water before use. A different area of each surface was tested each different time tests were made, but all swabs used in any one test were taken from the same area.

The order of taking the swabs, rubbed different numbers of times on the same area, is shown on the second and third days in the following tables, which summarise the results of these experiments:

Results from cotton.

Area tested	Time of drying	No. of swabs	How taken	Result		
				Positive	Negative	Contaminated
No. 1	3 hours	2	Rubbed repeatedly	0	2	0
,, 2	1 day	2	Each rubbed 8 times	2	0	0
,, 3	2 days	1st set 2	,, ,, 1 ,,	2	0	0
,, 3	2 ,,	2nd ,, 2	,, ,, 2 ,,	1	0	1
,, 3	2 ,,	3rd ,, 1	,, ,, 4 ,,	1	0	0
,, 3	2 ,,	4th ,, 1	,, ,, 6 ,,	1	0	0
,, 4	3 ,,	1st ,, 1	,, ,, 6 ,,	1	0	0
,, 4	3 ,,	2nd ,, 1	,, ,, 4 ,,	1	0	0
,, 4	3 ,,	3rd ,, 2	,, ,, 2 ,,	2	0	0
,, 4	3 ,,	4th ,, 2	,, ,, 1 ,,	2	0	0
,, 5	8 ,,	6	,, ,, 1 ,,	0	4	2
		22		13	6	3

Results from wood.

No. 1	3 hours	2	Rubbed repeatedly	2	0	0
,, 2	1 day	2	Each rubbed 8 times	1	1	0
,, 3	2 days	1st set 2	,, ,, 1 ,,	0	2	0
,, 3	2 ,,	2nd ,, 2	,, ,, 2 ,,	2	0	0
,, 3	2 ,,	3rd ,, 1	,, ,, 4 ,,	1	0	0
,, 3	2 ,,	4th ,, 1	,, ,, 6 ,,	1	0	0
,, 4	3 ,,	1st ,, 1	,, ,, 6 ,,	0	1	0
,, 4	3 ,,	2nd ,, 1	,, ,, 4 ,,	0	1	0
,, 4	3 ,,	3rd ,, 2	,, ,, 2 ,,	2	0	0
,, 4	3 ,,	4th ,, 2	,, ,, 1 ,,	2	0	0
,, 5	8 ,,	6	,, ,, 1 ,,	0	5	1
		22		11	10	1

From this table it is seen that, omitting the eighth day results, attempts to obtain positive results from the cotton were successful in three out of the first four tests, while some swabs were positive in each of the first four tests made from the wood.

(3) *The Transmission of the Bacilli by Persons suffering from Atypical Forms of Diphtheria.*

That diphtheritic infection may pass from patients to healthy individuals by the means which have been mentioned has long been recognised, and therefore scarcely needs illustration. The more interesting question, however, of the danger to the community arising from the presence amongst the normal population of more or less healthy infected individuals must be discussed at some length. Many of these persons have probably been infected from patients, before the development of clinical signs, by the means by which they themselves spread the infection. These more or less healthy individuals, who have become infected with diphtheria bacilli and are therefore possible factors in spreading the disease, may be conveniently divided into three groups according to the classification of Watson Williams (1905).

Group 1. "Patients who afford none of the usual clinical indications of diphtheria, are not definitely ill, and yet are found to be anaemic, have increased pulse frequency, are poorly, in association with nasal catarrh, membranous rhinitis, faucial redness, and slight subacute tonsillitis, otorrhoea, sores, etc., which on bacteriological examination prove to be diphtheritic."

Group 2. "Cases with any of these diphtheritic lesions, but with no general symptoms of ill health." (Group 3, see p. 314.)

That persons belonging to group 1 are capable of spreading the disease, and even of causing considerable outbreaks, has been well recognised.

Owing to the fact that many of the accounts do not differentiate between these groups it is at present difficult to determine, whether the members of group 2 are less dangerous than those of group 1, or not. Williams considers that they have less frequently given rise to epidemics.

Some noteworthy examples are, however, quoted in the following pages, which either gave rise to a considerable number of cases, or caused infection after long periods of time.

(a) *Nasal catarrh.*

Cobbett (1901, p. 231) records as follows a remarkable instance of an outbreak traced to a child at first apparently suffering from nasal catarrh. "While the actual origin of the outbreak has not been cleared up, there is no doubt about the way in which the infection became distributed: on October 23rd, G. N. was visited among other infants attending this school. He was found to be having tea with his brother and sister, and was in very good spirits, though he looked rather pale, and appeared to be suffering from nasal catarrh. His mother said he had had a 'stuffy cold in his head' for about three weeks. During this time he had been regularly attending the school. Bacteriological examination revealed diphtheria bacilli in great numbers in the nasal discharge, and after his removal to the hospital membrane was seen in his nose. His father, mother, sister and brother, all the members of this family, were found to have diphtheria bacilli in their throats. From three of these (including G. N.) cultures were isolated and proved to be virulent. No less than seven of the nine male members of his school class suffered from diphtheria before October 23rd."

Burnett (1900) also gives an instance of the discovery in the nasal discharge of a boy, supposed to be suffering from a severe cold, of diphtheria bacilli, which lingered for three months. After two negative bacteriological examinations he was allowed to return to school. Shortly afterwards a fatal case of diphtheria occurred, and on examination diphtheria bacilli were again found in the boy's nose.

Park and Beebe (1895, p. 41) mention an interesting case. "A child was admitted into a hospital ward in an anaemic condition and with a chronic coryza. Five days later four children in his neighbourhood developed diphtheria. Two of these died. In seeking the cause of the diphtheria, suspicion was directed to the child by a slight nasal discharge. Bacteriological examination showed this secretion contained many diphtheria bacilli. On further examination it was found that the child came from a family in which three weeks before there had been a case of diphtheria."

Newsholme (1904) quotes the following example. F. T., aged four, developed diphtheria on November 16th, and it was found that on November 7th, his brother I. T. had suffered from headache followed by nasal discharge. He had continued to attend school until the 18th, and infected three other children in the same class, who failed with the disease, two on November 14th, and one on the 19th. Diphtheria bacilli was found in I. T.'s nose.

(b) *Fibrinous, or membranous rhinitis*[1].

Cobbett (1901) observes that "virulent diphtheria has been but seldom observed to be contracted from contact with a case of membranous rhinitis," and some observers have considered that the virulence of the bacilli isolated from such cases is low, and that cases of this disease are apt to give rise to others of the same kind. Some statistics on this latter point are given elsewhere (Chapter X) which show that a very large proportion of the bacilli isolated from these cases are fully virulent, and several cases of clinical diphtheria occurring through contact with persons suffering from membranous rhinitis have been recorded.

---

[1] Cobbett's case quoted under the heading of Nasal Catarrh, although no membrane was at first noticed, perhaps ought to be more properly included amongst this series.

Dowson (1895) gives a very interesting example with the following history. "L. H. and S. H. went to a children's party on December 17th, 1894, at the house of a lady who had a tendency to suffer from sore throat, and who about three weeks before the party was under the care of a medical man, the father of L. H. and S. H., for a somewhat indefinite ailment of the throat, attended with slight difficulty in swallowing. Both children were kissed by this lady, and on December the 19th, L. H., a healthy boy of 4½ years, had a slight nasal catarrh, but was apparently quite well otherwise. On December 28th, his right nostril was noticed to be blocked by a tough white membrane which, when detached, did not cause bleeding, although an abraded vascular surface was exposed. On January 2nd, S. H., the sister, aged six years, was noticed to be affected in a similar manner in the left nostril." Virulent diphtheria bacilli were isolated from these membranes. "At the end of two months, so very few bacilli, and these chiefly involution forms, could be recovered from the children's noses by culture methods, that they were considered harmless to others, and the fairly strict quarantine, which had been observed, was removed. Active local antiseptic treatment had been employed during the whole time. At the end of May, the mother of the children, after playing with and kissing the boy to a quite unusual extent, complained of sore throat, which on inspection proved to be a membranous pharyngitis presenting all the appearances of true diphtheria. This was confirmed by inoculating a culture tube from the membrane, the resulting growth being almost a pure culture of the diphtheria bacillus. It was at first sight thought that the mother's attack was due to some oversight in disinfection, but an examination of the children's noses showed that membrane resembling that first observed, was again present." Diphtheria bacilli were again found.

Ravenel (1895) gives an account of several children, who became infected from a child suffering from membranous rhinitis, produced by virulent diphtheria bacilli. The first child, who remained quite well except for a sensation of discomfort in the nose, had himself been infected by a child who only complained of a sore throat. The former gave rise to membranous rhinitis in three children, one of whom later developed pharyngeal diphtheria, and to two cases of the latter disease.

Cultures made from all these cases showed diphtheria bacilli. Wolff (1905) also quotes a case of true diphtheria apparently infected from a case of membranous rhinitis.

On the other hand, a case of membranous rhinitis has not infrequently been observed to give rise to another of the same kind. The cases just quoted from Ravenel are examples, and the same observer also gives another instance in which a mother and three children all suffered from this complaint. Abbott (1893) found two children affected in the same family, and Concetti (1892) obtained in two cases a history of direct infection from one to the other. Since that time several other instances of two or more members of the same family suffering from membranous rhinitis have been described. Lieven (1891) reported a case of membranous rhinitis from which he obtained an organism that when introduced into the noses of other children by means of tampons caused a similar condition in them.

(c) *Tonsillitis.*

Bissel (1902) relates a case in which a child, who had had a mild sore throat two months previously, went to pay a visit. Diphtheria occurred in the household visited, and was transmitted by this child as far as could be ascertained. He adds that many such fully authenticated cases have occurred in the city of Buffalo. Berry (1900) gives an account of a prolonged outbreak apparently transmitted through children suffering from sore throats. In the London Orphan Asylum a case of diphtheria was introduced amongst the girls (200) on February 25th. A series of mild sore throats followed till March 27th, when another case of diphtheria occurred and three children with sore throats were found to be harbouring diphtheria bacilli. These were isolated. Further cases of sore throat followed till April 30th, when five cases of diphtheria occurred. At this time there were 76 cases of sore throat. Dr Washbourn examined all the children with abnormal conditions of the throat and found diphtheria bacilli in 17. These were isolated and not allowed to return to school until free from the bacilli. From this time no further cases of sore throat or diphtheria occurred. Newsholme (1904) gives an instance of a child who was ill for one day with sore throat and headache. Five days later his sister developed diphtheria. This led to a swab being taken from his throat, from which diphtheria bacilli were cultivated.

Goodall (1896) quotes a case in which diphtheria was apparently transmitted by a patient several months after recovery from a mild attack. A nurse had a very mild attack in January and was isolated for several weeks because the bacilli persisted. When free she was put in charge of a ward of mixed diphtheria and scarlet fever. In the autumn she went home to a village in Oxfordshire, where there had been no diphtheria for a long period, and almost immediately her sister and niece developed diphtheria.

(*d*) *Otorrhoea.*

Newsholme (1904) gives an interesting example of infection through a case of otorrhoea. A baby, nine months old, was apparently infected from its sister who developed typical diphtheria. Except for the ear trouble the baby remained perfectly well, but infected another child, who suffered from pharyngeal diphtheria. At the time the other child became ill a pure culture of diphtheria bacilli was obtained from the ear discharge about a month after its commencement.

The writer (Graham-Smith, 1904) has also observed a small outbreak in which two persons were attacked with clinical diphtheria, and five became infected with virulent diphtheria bacilli, apparently by contact with a patient suffering from otorrhoea in whose discharge virulent diphtheria bacilli were found.

(*e*) *Other lesions.*

Newsholme (1904) quotes a case in which enlarged cervical glands were the only clinical evidence of a pathological condition of the throat. N. W., aged three, and his brother S. W., aged two, developed diphtheria on October 20th and 25th, respectively. Inquiry into these cases led to the discovery that the brother G. W., aged five, had come home from school with enlarged glands on October 7th. Diphtheria bacilli were found in his throat on October 25th.

(4) *The Transmission of Diphtheria by Healthy Contacts.*

Williams' third group consists of healthy infected contacts or "persons who present no local lesions and no departure from normal health, but in whom diphtheria bacilli have been found by culture tests." Williams says of this group that "it has yet to be shown that such infected contacts are liable to spread diphtheria or are actively infectious till they develop local symptoms." In support of this view he quotes instances in which healthy infected contacts were allowed to mix with the non-infected persons in institutions without ill effects. Diphtheria, however, is usually most prevalent at seasons of the year when "colds" are common, consequently a member of this group may at any time by developing a "cold" be converted into a member of group 2. Even if Williams' opinion is correct, it is therefore almost as necessary to guard against the possibility of the spread of the disease by members of group 3 as of group 2. There are, however, several examples to be found in the writings on this subject, which indicate that perfectly healthy infected contacts are capable of spreading the disease, and careful observation could probably multiply them. The writer has come across seven instances in which there could be but little doubt that the disease had been conveyed by such persons, and some in which the proofs were convincing. In one instance a girl aged 13 was admitted into a general hospital for an operation on the knee. Shortly after her admission a small outbreak occurred in the ward owing to the introduction of an unsuspected case. On the day before her discharge cultures were obtained from this girl, which showed the presence of a few colonies of diphtheria bacilli, which were later proved to be virulent. On the urgent request of her parents, however, she went home to a small village in which no cases of diphtheria had occurred. About a fortnight after her return, diphtheria broke out amongst the members of her family, of whom three suffered from the disease. Although the girl remained in perfect health subsequent cultures showed the presence of numerous colonies of diphtheria bacilli.

Bugbee (1904) gives the following example: A young woman, six weeks after an attack of diphtheria, came to reside at Brooklyn with a family consisting of two adults and two children. Six days later one adult and one child developed diphtheria. The grandmother of the healthy child came to attend to it and was in the house for four weeks, but did not see the diphtheria patients. When the grandmother returned home to Vermont two members of her family developed diphtheria within a week. From her throat a pure culture of diphtheria bacilli was obtained.

Park and Beebe (1895) quote an instructive case. "In a family of eight children one sickened of diphtheria and a second child, a baby, was sent to a neighbour. The next day cultures showed that this baby, as well as two of the other children, all of whom were apparently healthy, were infected with diphtheria bacilli. The three apparently healthy but infected children, as well as the sick one, were at once quarantined, but already one of the family, to which the baby had been sent, had contracted diphtheria from it."

Hutchens (1906) records a noteworthy example of the conveyance of diphtheria by a perfectly healthy contact. "One of the pupils at a private boarding school contracted diphtheria. As soon as the true nature of the disease was recognised, the throats of all the inmates of the establishment, some thirty in all, were examined. Diphtheria bacilli were found in the cook's throat and in no one else's. On being questioned she admitted having visited a neighbouring town some three or four days previously, and, while there, had been in contact with a case of diphtheria. These statements were investigated and found to be correct. There was no diphtheria in the town in which the school was situated, and the cook herself was in perfect health."

White (1901) also recounts an instance of the spread of diphtheria by perfectly healthy contacts. Four persons, continually exposed to a child which harboured diphtheria bacilli, were examined three months after the latter had recovered, and diphtheria bacilli were found in the throats of two of them. About this time two children were exposed to these persons for a day or two, and one of them on going home immediately developed diphtheria. No other source of infection could be traced.

Cuno (1902) investigated an outbreak which occurred first in one and then in another ward of a hospital. On careful examination it was discovered that one nurse had virulent diphtheria bacilli in her throat, and the outbreak was found to have followed her removal from the one ward to the other.

Newsholme (1904) also gives an instance of the spread of the disease by a healthy infected contact. F. W., aged six, attended until March 13th a school in which cases of diphtheria were occurring at the time. She was kept at home because she had ringworm, but is stated to have had no cold or sore throat. On March 19th, her sister E. W., aged 4½, failed with diphtheria. E. W. had not been attending school, and had been kept strictly indoors owing to a recent attack of chicken-pox.

Although no bacteriological evidence is mentioned, the following case related by Newsholme (1904) is of interest. During an outbreak of diphtheria a girl, although she sat next to another who attended school for several days while suffering from an attack of unrecognised diphtheria, remained perfectly well. Within a few days a young man who lodged at the former's house developed diphtheria. He had only one meal in the house which was brought up to him by this child or her sister.

One of the most interesting cases of the transmission of diphtheria by non-sufferers is that recorded by Peck (1895). The first case of the series was that of R. H., aged 45, who lived in house A, and began to be ill on October 12th, 1894. "The case was typical and was followed by double drop-wrist. No other persons in this house suffered from sore throat." The next case occurred in house B, which was half a mile distant from A. "There was no communication between the houses till December 26th, when the occupier began to work for R. H. Case 2, E. J., aged 7½ years, had a sore throat on or about January 2nd, 1895. The case was typical and followed by paralysis of both legs. The patient died of cardiac syncope. Case 3, E. J., aged four years, began to be ill on February 6th. This was a typical case and resulted in death from asphyxia. Case 4, J. J., aged 12, had a sore throat on February 16th. The case was typical and followed by dimness of sight and strabismus. Bacteriological examination of cultures showed the presence of large numbers of diphtheria bacilli." All these persons were members of one family. The next case occurred at house C., which was 1¼ miles distant from A and B. "R. J., aged 18, brother of the last three patients, worked here from January 15th. His mother stated that he was at home for a few minutes on the days his sisters were buried, but at no other time. He himself admitted having been at home on each Sunday to change his shirt, which he did in the room occupied by the patients. He stated that he did not suffer from sore throat at any time, but bacteriological examination showed typical diphtheria bacilli in large numbers to be present in his throat. There is no history of his ever having had diphtheria. Case 5, J. C., aged 18, began to be ill on February 26th. He worked on the same farm as the last youth, and slept with him. He went home to house D on February 27th, being then 'full of cooth,' and suffering from sore throat and unable to work. Typical diphtheria bacilli were present in large numbers on March 16th." House D was nearly two miles distant from all the other houses. "Case 6, J. C., aged 13, slept with the last patient, his brother. On March 8th he was seen for the first time by his medical attendant and was then dying. No bacteriological examination was made. Case 7, E. C., aged three, sister of the last two patients, began to be ill on March 12th. The case was a typical one, and an abundance of typical diphtheria bacilli was found on bacteriological examination."

"House B was probably infected by the father of the family, who himself did not suffer from the disease. His son R. J. did not suffer from the disease, though he was proved to have its germs in his throat, but he was the means by which it was carried to house C, whence it was taken to house D."

Cases of infection carried by patients long after they have recovered from the disease, and appear to be in perfect health, have frequently been recorded. Such persons are in the same condition as healthy individuals carrying virulent diphtheria bacilli and might reasonably be included in this group, though in most accounts they are separately considered.

It is only those persons who are infected with virulent diphtheria bacilli, who are apparently capable of transmitting the disease, since there is little or no evidence to show that persons harbouring non-virulent diphtheria bacilli can infect others with virulent bacilli.

The Mode of Transmission of Bacilli from unrecognised Cases and healthy infected Contacts to other Persons.

Instances occur, probably not infrequently, in connection with unrecognised cases, and healthy infected contacts, in which the bacilli are conveyed to others in saliva through coughing or sneezing. The kissing of babies and children as a means of spreading the disease has been particularly insisted on by Hewlett and Murray. These methods of infection can only affect a small number of very close contacts, mostly limited to the families or intimate friends of the infected children.

In schools, however, the passage of sweets, pencils, pens, slates, etc., from one child to another, and especially the habit children have of placing their fingers, and such articles as pencils in their mouths, explain the rapid spread of infection in such institutions; and the absence of these habits in adults may to some extent account for their relative immunity from the disease.

Some remarkable examples of transferences by these means are given out of the large numbers which might be quoted:

Cobbett (1901, p. 247) gives two examples. "The day before B. C. was taken ill he had spent the evening with some neighbours. Four of these were examined for diphtheria bacilli with the result that they were found in two boys of 16 and 20, but not in a baby, nor yet in a sister of 14. On further inquiry it was discovered that B. C. had played with one of these boys at parlour cricket and each had taken it in turn to score with the same pencil, which doubtless often found its way into their mouths. It is quite clear then how the bacilli found their way from B. C. to this boy. From him they easily found their way to his brother, for the two boys slept in one bed." Cobbett (1901, p. 232) also traced the spread of the disease amongst certain school children in a class to the hours during which slates were used. This class consisted of nine boys and seven girls. It was notable that while seven of the nine boys of this class suffered from diphtheria, only one of the seven girls in it was affected, and one other was discovered to be harbouring a virulent diphtheria bacillus. For almost all the lessons the boys and girls of this class were mixed indiscriminately, and according to their school mistress they played together out of school hours. The only explanation of the unequal incidence of the disease upon the boys and girls was that twice a week the girls were separated from the boys to do needlework, whilst the latter had a drawing lesson. It can scarcely be doubted that it was during this drawing lesson, when slates were in use, that infection was distributed. One of the boys attended the class for three weeks while suffering from unrecognised nasal diphtheria.

In this connection Bond (1898) remarks that he has ascertained that in certain schools each child does not have its own slate, and that as a means of cleaning their slates licking is common.

Wesbrook (1900) remarks that "in watching children at play it does not take long to be convinced of the almost innumerable opportunities afforded for the exchange of the bacterial flora of mouths and noses," and later states (1905) that "the experience of Minnesota would seem to point decidedly to the conclusion that diphtheria infection is transmitted usually by almost direct exchange of the flora of the nose and mouth."

The Conveyance of Infection by other means than Personal Contact.

As compared with personal contact the other means by which diphtheria may be spread are, with the exception of infection through milk, of little practical importance. Infection is recorded to have been conveyed by infected articles, milk, food substances, animals, and birds, and by the agency of air currents, drains, and soil.

(1) *Infected articles.* Instances of infection by means of infected articles which may pass from mouth to mouth, practically amounting

to direct transmission, as well as examples of the conveyance of diphtheria by musical instruments, etc., long after their infection, have already been quoted. Experimental evidence of the cultivation of diphtheria bacilli from articles closely connected with patients, as well as evidence showing that diphtheria bacilli may remain alive for long periods in a dried condition, have also been given.

Many other articles may become soiled by diphtheritic excretions or saliva containing virulent bacilli and convey the disease, but attention ought to be especially directed to speaking tubes and to cups or glasses from which several individuals drink in succession, more particular those attached to public fountains.

(2) *Food substances.* Experimental evidence of the time diphtheria bacilli may remain alive and retain their virulence in some food substances, including water, has already been given (p. 172). Certain of these substances, such as sweets and fruit, which often pass from one child to another, frequently convey infection in the same manner as pencils, etc. Park and Beebe (1895, p. 51) traced a group of cases to a sweet shop. "The child of a man who kept a candy-store developed diphtheria; there were four other children in the family, and these were in no way isolated from the sick, yet none of them developed diphtheria; but the children who bought candy at the store, and other children coming in contact with them at school, developed diphtheria. The secondary cases ceased to develop as soon as the candy-store was closed."

No widespread epidemic seems to have been reported which was spread by the agency of any infected food substance except milk. Vincenzi (1898), however, on examining the holy water in churches during an epidemic found amongst other bacteria, diphtheria bacilli. He proved their identity by culture and virulence tests.

(3) The evidence relating to the dissemination of the disease by *animals* and *birds*, is fully given elsewhere (Chapter VII).

(4) *Air-borne infection.* Welch (1891), writing 15 years ago, said that "diphtheria is one of the infectious diseases the germs of which may be taken into the body by the inspired air," and up to the present time examples are occasionally quoted of apparent air-borne infection.

It must, however, be pointed out that diphtheria bacilli have seldom, if ever, been found in the air of wards or rooms occupied by diphtheria patients, in the few attempts which have been made to investigate this problem.

Wright and Emerson (1894), although they found diphtheria bacilli on shoes, brushes, etc., twice examined the air of the ward with negative results. Sudeck (1898) exposed plates in rooms, some occupied by healthy persons and others by diphtheria patients, and obtained some colonies of diphtheria-like bacilli, but never found true diphtheria bacilli.

Cobbett (1904) says he has been unable to find diphtheria bacilli on plates exposed in diphtheria wards except in situations in which they could easily be contaminated by the patients, and Hill (1902) also in a large number of experiments only appears to have found bacilli in similar situations (see p. 308).

Though it cannot be denied that air-borne infection may occur, both the experimental and the more recent clinical evidence point to its being a very rare mode of dissemination.

(5) *Dust.* That the dust found on the floors of hospital-wards may contain virulent diphtheria bacilli has been proved by the researches of Wright and Emerson (p. 308), and the power of the diphtheria bacillus to survive for long periods in dust on dry surfaces has been experimentally demonstrated by Germano (1897), Hill (1902) and others.

The possibility therefore of infection by means of floating particles of dust stirred up by sweeping, or otherwise, cannot be denied. Conclusive evidence of infection by this means has not been obtained, though some instances are given in the literature, which might be attributed to this cause, as, for example, of persons developing the disease after moving into houses lately occupied by patients. Some of the cases attributed to aerial infection may also have been due to dust.

(6) *Soil*[1]. References are occasionally to be found relating to the origin of diphtheria from polluted soil, but very little evidence is to be found in support of this view, although Leighton (1901) has shown experimentally that diphtheria bacilli can remain alive in moist warm clay for 18 days. Sharp (1896) stated that he found organisms morphologically like diphtheria bacilli and having similar colonies in two soils obtained from a locality in which diphtheria existed. No further tests to prove their identity appear to have been made.

(7) *Drains and sewer gas.* The belief is extremely widely held, particularly in Great Britain, that sewer gas is the predisposing cause of diphtheria, and scarcely a case occurs but it is attributed to "drains"

---

[1] The condition of the soil in regard to dampness and the exhalation of gases may affect the incidence of diphtheria by lowering the general health (see p. 69).

by the friends, and frequently by the medical men. Individual cases and even outbreaks have been frequently attributed to decaying manure and refuse heaps.

For example, Sutton (1894) states that in the parish of Upchurch large quantities of refuse from London dust-bins are brought by barges, and deposited on the quay. "These masses lie mouldering for weeks and the noxious fumes are carried in the direction favoured by the wind." At one time five cases of diphtheria occurred, and Sutton considered that these were infected by the foul gases. He concludes with the following observations:

"After due inquiry as to the origin of the first and subsequent cases, I came to the conclusion that these cases of diphtheria originated in the deleterious emanations generated in such immense volumes and were air-borne, especially as there was an utter absence of proof, or even suspicion, of direct personal contagion and no evidence pointing to schools as the cause. I am fully of the opinion that this disease originates with insanitary conditions, especially foul nuisances."

On the other hand Delépine (1904) recently investigated an outbreak attributed to an effluvium nuisance from a tannery, but found on careful inquiry and examination that the infection had been brought into the locality by a diseased person.

Many of these illnesses, which are mistaken for diphtheria, and which apparently result from the inhalation of foul gases, are, however, not associated with diphtheria bacilli. Diphtheria bacilli have never been found in drains or sewer gas, or in refuse heaps, and there is no bacteriological evidence to show that the emanations from the latter can originate true diphtheria; nor is there evidence that bad drains and insanitary environment can ever convert non-virulent into virulent bacilli. Shattock (1898) experimented on this question and found that it was impossible to raise the virulence of lowly virulent bacilli by cultivating them in sewer air, even after two months.

Hutchens (1906) gives an interesting example of apparent infection due to a bad system of drainage, which was subsequently proved to be caused by infected milk (see p. 325).

(8) *Laboratory infection.* Accidental infections in laboratories have from time to time been recorded, but are very rare.

Milk Epidemics.

In 1878 Power[1] brought forward evidence that diphtheria might be spread by means of milk. Between 1878 and 1882 several epidemics occurred in which the milk appeared to be the principal source of infection. The first milk epidemic following the discovery of the diphtheria bacillus occurred in York Town and Camberley in 1886 and was investigated by Power[1], and Klein's experiments, already quoted (p. 282), were undertaken in connection with this outbreak. Several outbreaks have since been recorded in which the milk supply has been the only source of infection in common amongst the patients, but in the great majority bacteriological evidence of the presence of the diphtheria bacillus in the milk, or in the throats of the milkers or their families, or in any lesions on the cows' udders is wanting. References[2] only to the most striking of these outbreaks are given, although the reports of some of them are extremely convincing and show that the outbreaks came to an end when the infected milk supply was stopped.

---

[1] *Local Government Board Report for* 1878.

Some typical examples of milk epidemics are here summarised in which some bacteriological evidence of diphtheritic infection amongst the milkers was obtained, as well as all those outbreaks in which diphtheria bacilli were actually isolated from the milk.

Appleget (1893) describes a severe epidemic apparently due to infected milk at Hightown, a place of 2,000 inhabitants. Although no bacteriological proof of the contamination of the milk is produced the outbreak has several features of interest. In the first week of the outbreak 28 persons were attacked, and of these 11 died. Milk was supplied to the town by six dairymen but the disease was confined to the customers of one of them. Investigation on the fifth day of the outbreak showed that the boy who washed the cans at this dairy had a sore throat with patches of membrane on the tonsils, and had been ill for several days previously. In one instance the adults in one house obtained their milk from this source, while the children obtained it from another. Two of the adults contracted the disease, but the children escaped. Only two cases occurred outside the village, one the daughter of the dairyman, and the other his niece.

---

[1] *Local Government Board Report for* 1886 (pp. 311–326).

[2] The following outbreaks are good examples of diphtheria apparently spread by milk.

Little Horton, Bradford, 1879 (H. Butterfield, *Brit. Med. Journ.*, 19. vi. 1880, vol. I. p. 953).

Kilburn and St John's Wood, 1879 (W. H. Power, *Local Government Board Report*, 1879).

Rugby, 1881 (G. Wilson, *Brit. Med. Journ.*, 3. ix. 1881, vol. II. p. 415).

Hendon, 1882 (W. H. Power, *Local Government Board Report*, 1884, p. 42).

Devonport, 1882 (H. F. Parsons, *Brit. Med. Journ.*, 5. v. 1883, vol. I. p. 876).

Canterbury, 1886 (Wacher, *Brit. Med. Journ.*, 21. viii. 1886, vol. II. p. 397).

Melrose and Malden, 1887 (J. S. Clark, *Brit. Med. and Surg. Journ.* vol. II. p. 100).

Enfield, 1887 (R. B. Low, *Local Government Board Report*, 1888, p. 123).

Croydon, 1890 (Philpot, *Brit. Med. Journ.*, 1891, vol. I. p. 470).

Worcester, 1891 (Thussfield, *Public Health*, 1891–2, p. 130).

Surbiton, 1891 (Coleman, *Public Health*, 1891–2, p. 158).

Glasgow, 1892 (Russell, *Brit. Med. Journ.*, 1892, vol. II. p. 432).

Limekilns, 1892 (Nasmyth, *Annual Report*, 1892).

Short accounts of most of these outbreaks are given by Swithenbank, H., and Neumann, G. (*Bacteriology of Milk*, 1903, pp. 346–351).

Howard (1897) investigated a more extensive outbreak of diphtheria at Ashtabula in which 100 cases were notified, of which 64 occurred within eight days. These 64 occurred in 49 widely separated houses, and their true nature was proved by the isolation of diphtheria bacilli from many of them. These cases were entirely confined to houses receiving their milk from one source. On inquiry at the dairy it was ascertained that one of the workers had recently suffered from a bad sore throat, and that this patient, while ill, had assisted at the dairy work. At the time of investigation no diphtheria bacilli were found either in the milk or the throat of this person.

Lee (1898) records an outbreak in which, owing to its sudden origin and the impossibility of the patients having come into contact with one another, the milk supply was immediately suspected. On investigating the farm from which the milk was obtained it was found to be in a filthy condition, and streptococci and bacilli morphologically resembling diphtheria bacilli were cultivated from the milk. The latter, however, could not be isolated. Further investigation revealed the fact that the nephew of the dairyman had diphtheria bacilli in his throat, and that a case of severe diphtheria had occurred there three weeks previously.

Biggs (1900) reports a somewhat similar outbreak at Ithica. The milk supply of all the families affected came from one dealer. On inspection all his premises and sanitary arrangements were found to be in good order. This dealer obtained some of his milk from a neighbouring farmer, and in consequence the farm was also visited. Several of the inmates were found to be suffering from sore throats, and cultures taken from their throats showed the presence of diphtheria bacilli. The milk supply was stopped from this farm, and the epidemic came to an end.

The bacteriological evidence in the case quoted by Chase (1900) is even stronger. Two children in a milkman's family developed diphtheria. All the members of the household were examined by cultures, but no diphtheria bacilli were found. Three weeks later diphtheria began to appear amongst the customers. On re-examination of the inmates of the milkman's house three men were discovered to be harbouring virulent diphtheria bacilli in large numbers. Up to this time two of these men had been employed in milking the cows.

Littlejohn (1900) reports a milk outbreak in Edinburgh and Liberton. At the dairy supplying some of the milk shops in these places a young man was found assisting at the business suffering from unrecognised diphtheria. As soon as the suspected milk was stopped the outbreak came to an end.

Although the bacteriological evidence is not conclusive, an outbreak of diphtheria at Leith recorded by Robertson (1905), apparently spread through the agency of milk, is of some interest. The outbreak coincided with the appearance of ulcers on the teats of the dairy cows, of which 19 out of 45 were affected. When all the diseased cows were isolated the outbreak ceased. Although diphtheria bacilli were found in the throats of most of the patients, swabs taken from the ulcers of the cows only showed a few doubtful organisms whose identity was never proved.

Hutchens (1906) cites an interesting example of the conveyance of diphtheria by infected milk. "A village school was re-opened after the vacation on a certain Tuesday; on the following Thursday evening the schoolmaster's child, aged three years, had a sore throat, which was proved by bacteriological examination to be diphtheria. The following morning three or four children in the village who had attended school on the three previous days also developed the disease. The schoolmaster's child had not himself been to school, neither had he been in contact with the other children. This child only arrived in the village on the Monday night, and there was no diphtheria in the place whence he had come. The school itself had long been notorious for its lack of drainage, and during the vacation a system of drainage had been introduced, but was not completed before the school was reopened. The surroundings both of the school and of the schoolmaster's house adjoining were most insanitary and offensive. This to the casual observer, was an undoubted instance of diphtheria due to 'insanitary surroundings,' and the outbreak was in fact locally attributed to this cause. Inquiries were now directed to ascertaining whether there were any cases of diphtheria in the neighbouring village. Some four or five were discovered. These could not be explained on the theory of direct contact, as they dated their illness from the same period as the school children. The question of milk supply was considered. It was then found that all the houses in which cases of diphtheria had arisen were supplied with milk from the same farm. A visit was paid to the dairy farm some miles from the village; it was there found that one of the milkers was complaining of a 'sore throat.' Swabs were taken from his throat and from the throats of all the other people living at the farmhouse. I found virulent diphtheria bacilli in three of these throats. Precautions were taken to prevent those harbouring the bacilli from having anything further to do with the milk, and no further cases occurred in the village. The milk supply from the farm to the village was not interrupted for a single day."

In the following instances virulent diphtheria bacilli were actually isolated from the suspected milk.

Bowhill (1899) isolated virulent diphtheria bacilli from suspected milk during an outbreak at Senghenydd.

Eyre (1899) isolated diphtheria bacilli from the milk supplied to a large school in which diphtheria was prevalent, and proved them to be fully virulent. He obtained them in all the 15 serum tubes sown from the cream obtained after centrifugalisation, but in only five out of 15 sown from the sediment. The examination of this milk was undertaken in connection with an extensive outbreak of diphtheria investigated by Goadby (1900) (see p. 186).

The small outbreak investigated by Dean and Todd (1902) has already been described. The bacilli were isolated from the milk and lesions on the cows' udders, and also from some of the patients, and proved to be virulent (see p. 286).

More recently Ashby (1906) investigated a milk epidemic, and found virulent diphtheria bacilli in the lesions of the cows' teats, but not in the milk (see p. 287).

Klein (1901) obtained a virulent diphtheria bacillus from one out of 100 samples of milk in London. No information could be obtained as to the source of infection.

Summary of Milk Epidemics.

Klein (1890) concluded from his experiments that under certain conditions diphtheria bacilli might be found in the milk as drawn from the udder, and pointed out that in certain outbreaks in which the milk was suspected the cows were suffering from lesions similar to those he obtained experimentally. Other observers have, however, failed to corroborate Klein's results, and the general opinion appears to be that in these cases the diphtheria bacilli are not derived from the cow. Virulent diphtheria bacilli have on four occasions been found in milk and twice, by Dean and Todd (1902) and Ashby (1906), in the naturally acquired lesions of cows' teats.

The experiments, however, of the former workers showed that the lesions were not due to the diphtheria bacilli.

It has been shown that unsterilised milk at ordinary temperatures is a suitable nidus for the multiplication of diphtheria bacilli (p. 172), and there can be no doubt that milk has been the vehicle for the contagion in certain outbreaks. In most of these it seems probable that it was infected at the dairy or at some other place before reaching the consumers. Dean and Todd point out that a ready means of infection of milk by persons harbouring diphtheria bacilli is the filthy habit of some milkers of spitting on their hands.

Diphtheria-like organisms in Milk.

The occurrence of diphtheroid organisms in milk, recorded by many observers, emphasises the necessity for very careful investigations, including animal inoculations, before a diagnosis of the presence of diphtheria bacilli in milk can be made.

Bergey (1904) isolated from milk, drawn under his observation directly from the udders of the cows, five strains of diphtheria-like bacilli, which in morphology belong to A_1, C_1, and D_1 of Wesbrook's types. They form small smooth gray or yellowish colonies on *serum* like those of the diphtheria bacillus, and on agar the growth is also diphtheria-like. Acid is produced in *glucose broth* and in *milk*. No visible growth occurs on *potato*, and no *indol* is produced. These organisms are non-pathogenic. He also found other organisms which resembled diphtheria bacilli less closely in morphology, produced pigmented growths and also differed in other cultural characters.

Klein (1901, p. 87) isolated from milk a diphtheroid organism, which he named *Bacillus diphtheroides*. This organism resembles the diphtheria bacillus in size, shape and clubbing. It does not stain very readily with the ordinary stains, but shows polar granules and retains Gram's stain. On *serum* it forms small, round granular colonies which at first slightly liquefy the medium and finally make it quite liquid. Growth on *agar* is slow. After three days the colonies are still very small, but after a week they have a dark centre and uneven edges. Very little growth occurs in *broth*. There is no growth on *gelatin*. *Milk* becomes acid and is coagulated.

Intraperitoneal or subcutaneous *inoculations* into guinea-pigs give rise to subacute abscesses containing thick yellow pus from which the organisms can be recovered. After subcutaneous injections the inguinal glands are especially affected, and after intraperitoneal the omentum, pancreas, and perinephric tissue.

McClure (1898) separated from milk a curved, banded bacillus which stained intensely at the poles, but did not retain Gram's stain. Under the microscope the bacilli had the characteristic arrangement of diphtheria bacilli. On *agar* it produced a considerable white, partly confluent, growth which later became yellowish. It grew rapidly on *gelatin* and produced a grayish white growth on *potato*. *Broth* was made cloudy and a white flocculent deposit was produced. *Milk* was turned acid in 48 hours and coagulated. A *mouse* injected subcutaneously died in 14 hours.

Eyre (1900) isolated from milk five organisms resembling the diphtheria bacillus "The interest of these observations lies in the fact that it would be quite easy to be deceived by the microscopical appearances of any one of these organisms." The organisms could on their cultural peculiarities be divided into three groups which he calls "Diphtheroid 1, 2 and 3."

Diphtheroid 1. *Morphology*. Pleomorphic bacillus of about the size of *B. diphtheriae*, showing segmentation of its protoplasm, metachromatic granules, and early involution forms, characterised chiefly by club shapes. It retains Gram's stain. On *serum* slender segmented bacilli showing a fair amount of metachromatism are seen. On *serum* discrete semi-transparent colonies occur, whitish in 24 hours, but taking on a yellowish colour in 48 to 60 hours, by which time many have coalesced into a thick layer. The growth afterwards becomes dry and powdery in appearance. On *gelatin* there is a good growth, white at first, later citron coloured. On *potato* there is a very slightly raised yellowish powdery growth. In *broth* a granular deposit is formed, but the medium remains clear. In *milk* a good growth takes place, but the medium remains unaltered. There is no acid, gas, or indol production. Non-pathogenic.

Diphtheroid 2. *Morphology*. Short pleomorphic bacillus showing segmentation of its protoplasm, metachromatic granules and early involution forms characterised chiefly by club shapes. Retains Gram's stain. On *serum* the bacilli resemble the "sheath form" of the diphtheria bacillus. On *serum* large, discrete, raised, spherical, opaque, grayish colonies are formed and the growth in some cases is confluent. On *agar* small, discrete, raised and transparent colonies are produced. After a few days' growth the colonies become faintly pink. On *gelatin* there is at first a good growth of discrete colonies which afterwards become confluent. The more superficial portions of the gelatin show a faint claret colour in about three days (the colonies remaining white or gray), which becomes deeper and tinges the whole of the gelatin in about a week. The colonies then acquire a faint rose pink tint. On *potato* a whitish shining growth occurs on the surface which becomes pinkish, and in three days the medium itself acquires a rose pink colour which becomes deeper. *Broth* becomes uniformly clouded, with a small amount of deposit at the bottom of the tube. A slight amount of acid is produced, but there is no gas or indol production. Non-pathogenic.

Diphtheroid 3. *Morphology*. Pleomorphic bacillus showing segmentation of the protoplasm and early club-shaped involution forms. On *serum* the majority of the bacilli stain badly. Some, however, stain well, show polar granules, and retain Gram's stain. On *serum* it forms a white, opaque, confluent growth, which appears as a film over the entire surface of the medium. On *agar* a good growth takes place in the form of raised, circular, white, opaque shining colonies, discrete at first, but tending afterwards to become confluent. On *gelatin* white or buff coloured very small colonies appear after several days growth. There is no growth on *potato*. In *broth* growth occurs as small confluent masses which fall eventually to the bottom of the tube. There is no acid, gas, or indol formation. No macroscopic change takes place in milk. It is non-pathogenic.

Eyre concludes by saying that from his investigations "It follows that it is practically impossible to diagnose the presence of the true diphtheria bacillus in a milk sample by microscopical tests alone, but that the identity of the Klebs-Loeffler bacillus can only be established by careful consideration of its biological and pathogenic characters."

Summary of Chapter VIII.

Patients suffering from diphtheria transmit the disease to others, either through the direct deposition of infected material on to the mucous surfaces or hands of their attendants or friends, or through the agency of infected linen, toys, etc. The extent to which the disease may be spread by patients suffering from clinically typical diphtheria depends on the measures adopted to secure complete and efficient isolation, and may be very great in those cases in which the arrangements for isolation are defective. Persons suffering from unrecognised diphtheria are a far greater source of danger to the community than those who develop the disease in a typical form, and are frequently the cause of extensive outbreaks. Such atypical forms of diphtheria may include nasal catarrh, membranous rhinitis, atrophic rhinitis, tonsillitis, acute and subacute, otorrhoea, and apparently simple catarrh. Other persons acquire the bacilli but develop no symptoms, but are nevertheless capable of infecting others in the same way, and of giving rise to diphtheria. Children suffering from unrecognised diphtheria and healthy children infected with diphtheria bacilli spread the disease mainly by means of articles which pass from mouth to mouth, such as pencils, toys, and cups. Personal infection through these agents is by far the most important factor in the dissemination of diphtheria, and is the only one which plays an important part in most epidemics.

Infected milk has apparently been the cause of some widespread outbreaks.

All other possible means of transmission appear to play a comparatively unimportant part. A few undoubted instances of infection through animals and of accidental infection from cultures are recorded.

Very little bacteriological evidence can be produced to support the alleged instances of air-borne transmission, or infection through soil, dust, drains, sewer gas or decomposing refuse.

CHAPTER IX.

BACTERIOLOGICAL DIAGNOSIS.

Direct examination of cover-glass preparations. Swabs. Inoculation of culture media. Choice of media. Temperature and time of cultivation. Methods of making preparations for microscopical examination. Smears. Preparations from separate colonies. Staining of fixed preparations. Significance of polar bodies. The recognition of diphtheria bacilli under various conditions. Methods of obtaining pure cultures. The distinguishing cultural and pathogenic properties of the diphtheria bacillus. Method of recording the morphological appearances. Unusual diphtheritic lesions of the mouth. Diphtheria-like organisms found in the mouth. Diphtheritic lesions of the nose. Diphtheria-like bacilli found in the nose. Diphtheria of the eye and diphtheria-like bacilli found in the eye. Diphtheria of the ear and diphtheria-like bacilli found in the ear. Diphtheria of the skin and diphtheria-like bacilli found on the skin. Diphtheria of the female genital organs and diphtheria-like bacilli found on them. Diphtheria of the male genital organs and diphtheria-like bacilli found in the urethra. Diphtheria of the anus and rectum. Diphtheria and diphtheria-like bacilli in the urine.

Direct Examination of Cover-glass Preparations.

For the purpose of rapid diagnosis, and as an aid in the examination of cultures, the direct examination of smears made from pieces of membrane or material from the surface of swabs has been greatly used. These smears, made on cover-glasses and allowed to dry, are fixed by rapidly passing through the flame or by the application of absolute alcohol, and are subsequently stained by Loeffler's methylene blue, or by Neisser's method, or some modification of it, and mounted. The general arrangement of the diphtheria bacilli, and their staining properties under these conditions, have already been described (p. 124). By means of smears a correct diagnosis of the presence of diphtheria bacilli can frequently be made in clinical cases of diphtheria, but even in these cases the subsequent examinations of cultures show diphtheria bacilli to be present in a considerable proportion of cases in which they had not been recognised in the smears. It follows, therefore, that very little trust can be placed on negative smear examinations, even in clinical cases, and none whatever in the case of convalescents, and contacts.

Again a positive diagnosis of the presence of diphtheria bacilli may not be confirmed by the culture test, since, whatever staining method may be used, other organisms are apt to be mistaken for diphtheria bacilli. Irregular, spindle-shaped bacilli and fragments of streptothrices, somewhat resembling true bacilli, are frequently met with in smears, and implicit reliance cannot be placed on Neisser's or other special stains since cocci and bacilli showing polar granules are of common occurrence.

The statistics of a carefully controlled set of experiments are quoted below (Mason, 1901):

Swab and serum culture both positive	37
Swab negative, serum culture positive	10
Swab doubtful, serum culture positive	7
Swab positive, serum culture negative	3
Swab and serum culture both negative	34
Swab doubtful, serum culture negative	6
Swab doubtful, serum culture no growth	2
	99

By culture tests 54 of these cases showed diphtheria bacilli, but only in 37 or 68 % was the swab diagnosis positive, and amongst the 45 cases negative by culture three (6·6 %) in smears showed organisms which were taken to be diphtheria bacilli, and eight (17·7 %) showed doubtful organisms. In this series including the doubtful cases, the smear diagnosis differed from the culture diagnosis in 28 %.

Hector (1905) examined 100 consecutive cases both by direct smears from the swabs and by cultures. In no case were diphtheria bacilli diagnosed in the smears and not subsequently found in the cultures. Cultures from 36 cases showed the presence of diphtheria bacilli, but they were only recorded as present in smears from 21 (58·5 %) of these.

Pugh (1905) using a special stain (p. 146) found diphtheria bacilli with definite polar bodies in smears made direct from the swab in 231 (57 %) out of 402 examinations. Cultures from all these cases were positive.

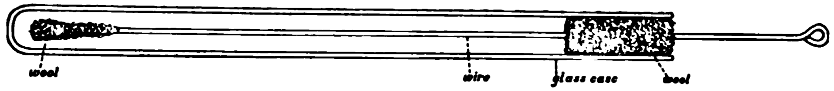
Beaton, Caijer, and Pakes (1901) and Cammidge (1901) believe that a trustworthy positive result can be obtained from the swab direct. Salus (1902) is satisfied if he finds diphtheria bacilli in one out of five smears from diphtheria cases.

Concetti (1900) advises the use of glass rods wrapped round with cotton wool impregnated with glycerinated glucose agar. One of these is rubbed on the throat, replaced in the tube, and placed in the incubator. After a few hours colonies develop and a smear is made from the rod and examined.

Smears are, however, of great use in differentiating Vincent's angina from true diphtheria, since the fusiform bacilli constantly associated with the former disease cannot be cultivated aerobically. *Bacillus fusiformis*, and the spirilla which frequently accompany it, can be readily recognised in smears, and attention directed to the probable cause of the disease, before cultures can be examined (see Chapter X).

Bacteriological Diagnoses by Means of Cultures.

Swabs. Swabs are now in general use for obtaining the material with which the culture tubes are inoculated. The swab consists of a small quantity of cotton wool wrapped round a stout wire, the end of which is either roughened or slightly flattened. The wires are placed in glass or metal cases, so that the whole may be sterilised. Sterilisation is generally accomplished by heating to 160° C. in a hot air steriliser for two hours. A convenient and cheap form of swab is illustrated below:



Platinum loops and various other devices have been made use of instead of swabs.

Swabbing. In taking a swab from the mouth care must be exercised not to touch the surface of the tongue or other parts, either when introducing or removing the swab. It should be rubbed on the surface of the membrane or exudate, or, if neither of these is present, over the diseased area, and immediately replaced in its case. Pus or discharge from the nostrils can also be collected on swabs.

In the case of contacts the swabs should be taken from any unhealthy areas that may be seen and also from the surfaces of both tonsils. Where exudation is seen from any of the crypts an attempt should be made to inoculate the swab with some of this material.

Occasionally it is possible to express purulent material from the crypts of the tonsil, when very little can be seen on the surface, by pressing the tonsil inwards with the fingers below the angle of the jaw and rubbing the surface with the swab.

Swabs should not be taken shortly after the application of antiseptics, as the growth of the bacilli on the culture media is thereby likely to be hindered or entirely inhibited.

Under certain circumstances it may be impossible to sow the swab on culture media for several hours. Under these conditions it is advisable to moisten the surface of the swab with sterile water or broth before it is taken.

Inoculation of Culture Media.

For the purposes of routine examinations swabs are usually sown on the sloped surfaces of serum tubes, each swab on a single tube. The general custom is to thoroughly rub the swab over the surface of the medium in such a manner that all parts of the swab come into contact with the surface. In certain cases where there is likely to be a very copious growth the writer has found that a very satisfactory method is to rub the swab thoroughly over one side of the slope and pass it lightly over the other. The result is that at least on one part of the slope more or less separate colonies can usually be found. For special investigations the swabs may be rubbed over the surfaces of several tubes in succession, so that under any circumstances discrete colonies may be obtained. For this purpose plate cultures may also be employed, or small pieces of membrane or material from the surface of a swab may be made use of to inoculate sterile salt solution or broth tubes, and cultures be subsequently made from these.

Choice of Media.

Loeffler's medium made from horse or ox serum has been most extensively used. Kanthack's serum has also been employed by several observers, and Cobbett's modification of Lorrain Smith's serum medium was used by Cobbett in his investigations at Cambridge (see p. 150). The writer has also used this medium in the examination of several thousand cultures.

Comparative tests between Cobbett's medium and Loeffler's have convinced the writer that diphtheria and Hofmann's bacilli grow equally well on both media, and that consequently owing to its transparency the alkaline serum is the most convenient for diagnostic work, since the separate colonies are more easily seen and their characters recognised, and it is simpler to obtain specimens from single colonies for microscopic preparations or the making of subcultures.

So far as the writer's experience goes media made from human exudates are unsatisfactory, as Hofmann's bacillus is apt to resemble the diphtheria bacillus more closely than when media made from ox serum are used.

Temperature of Growth.

The cultures are generally incubated at 37° C., but various temperatures have been advocated between 35·5° C. and 37° C. Those who advocate the lower temperatures think that diphtheria bacilli alone develop polar bodies within a given time (18 to 20 hours), and may therefore be diagnosed by their presence (see p. 142).

Time of Cultivation.

The great majority of observers examine their cultures after 12—18 hours' growth, and most of them do not re-examine them after further growth. As has already been pointed out diphtheria and Hofmann's bacilli generally develop recognisable colonies in this time, whereas the majority of organisms found in the throat do not show visible growth on serum after this period of incubation. Certain cocci and bacilli do, however, form colonies within this period.

Methods of making Preparations for Microscopical Examination.

(1) *Smears made after a few hours' growth.* Ohlmacher (4. v. 1895) has recommended scraping the surface of the culture medium with a platinum loop after four hours' incubation at 37° C. He states that under these conditions "no trouble will be experienced in finding plenty of organisms upon which to base a satisfactory diagnosis."

The smear on the cover-glass must occupy a very small area in order that the organisms may not be too widely scattered and consequently missed. This method was advocated purely for the purpose of obtaining a very rapid diagnosis by culture.

(2) *Smears made from the surface of the culture after* 12—18 *hours' growth.* Many observers prepare smears on the cover-glasses by scraping material from the surface of the cultures. This method of obtaining preparations regardless of the separate colonies has been described earlier (p. 213). For the purpose of scientific research the results obtained from such preparations are obviously useless, since nothing but the morphology of bacilli inextricably mixed from various colonies can be determined.

It is impossible to state whether the various types found came from separate colonies or were present in single colonies. Further, if the whole surface of the tube has been scraped, it becomes hopeless to obtain pure cultures and test the accuracy of the diagnosis, and a second examination of the culture after further growth cannot be made. It necessarily follows that those who habitually employ this method give their diagnosis on certain fixed conceptions of diphtheria and other bacilli. They probably neither prove nor disprove the value of their diagnoses, and the errors are likely to be multiplied and perpetuated.

An example of the effects of such proceedings may be taken from Pl. XII, fig. 5. In this culture some of the larger colonies have been produced by Hofmann's bacilli and others by cocci, while the smaller colonies are those of the *B. coryzae segmentosus.* A smear from such a culture would probably lead to a diagnosis of diphtheria, since the latter organism (Pl. XIV, fig. 1) bears a striking resemblance to the segmented diphtheria bacillus. It would also be stated that Hofmann's bacilli occurred in conjunction with diphtheria bacilli. In other cases bacilli differing from diphtheria bacilli in every characteristic except morphology must be diagnosed as diphtheria bacilli. A more careful method at once obviates these sources of error. Even for the most superficial of routine examinations there is nothing to recommend the use of such smears except the saving of labour and time at the expense of accuracy.

(3) *Preparations made from separate colonies.* For the purpose of the routine diagnosis of diphtheria, preparations from separate colonies can be made with nearly as much ease as smear preparations, and the results are infinitely more satisfactory and reliable, and, for the purpose of obtaining statistics of scientific value or the examination of contacts or convalescents, are the only ones which are trustworthy. Several preparations from different colonies can be made on one cover-glass, and under these conditions can be examined faster than smear preparations. In the latter it is necessary to thoroughly examine the

whole smear in order to determine the absence of any given organism; in the former an examination of any part of a preparation from a single colony suffices. Preparations from single colonies are made in the following manner: the top of a colony is touched with a sterile needle, which is then dipped in a capsule of water and drawn two or three times along the cover-glass so as to produce a streak. The same process is repeated with different colonies until several lines (5 or 6), composed of micro-organisms from separate colonies, are made in parallel series on the cover-glass.

It might be thought that the water would become so contaminated that the preparations from the various colonies would eventually show several kinds of organisms. In practice, however, this is not the case. For greater accuracy minute drops of water may be placed in a row on the cover-glass and the inoculated point of the needle dipped in one of these before the streak from it is made. In all cases streaks should first be made from diphtheria-like colonies, and then from colonies more or less resembling them. Streaks from colonies widely differing from those of the diphtheria bacillus are made still later. If the examination of the diphtheria-like colonies does not reveal diphtheria bacilli several typical colonies from various parts of the tube should be examined. In all cases it is desirable as far as possible to examine all dissimilar colonies; since many of the organisms morphologically resembling diphtheria bacilli produce colonies totally different in appearance.

It occasionally happens that in crowded cultures the colonies are so small and closely aggregated that separate colonies cannot be examined. In these cases streaks may be made in the manner described from groups of similar colonies.

This method has numerous advantages over the smear method. Firstly, the colonies may be examined and the bacilli producing the various types recognised. In this way the writer has on several occasions been able to give a satisfactory diagnosis on organisms, which, from their microscopic appearance alone, might have caused trouble, but which formed colonies entirely different from those of the diphtheria bacilli. Further, the various types of bacilli present in single colonies can be determined, a matter of some interest in view of the assertion that several types of diphtheria bacilli may be met with in single colonies. The staining reactions of the various organisms forming different colonies can be observed, which is impossible in smear preparations. Colonies of doubtful bacilli can be easily picked out for making subcultures and proving their identity.

This method allows also of the culture being further cultivated and the separate colonies being again examined after a longer period of growth.

Although in typical cases of diphtheria little advantage is likely to be gained by the latter proceeding except when slow growing varieties of the diphtheria bacillus are present (p. 246), or the previous application of antiseptics has hindered development, yet in the case of contacts and convalescents, as far as the writer's experience goes, the second examination frequently reveals isolated colonies of the diphtheria bacillus and also of Hofmann's bacillus, which had either been overlooked owing to their small size, or had not developed at the previous examination. This is especially the case when the patients are being constantly treated with antiseptics in order to hasten the disappearance of the bacilli. Such bacilli would be in the majority of cases entirely overlooked in general smears since a single minute colony contains but few bacilli, and the probability of not finding them in the smear, or of their not being included, is very great. During the last three years the writer has, as a routine practice, made a second examination of all cultures in which diphtheria bacilli were not originally found, and has discovered diphtheria bacilli in the second examination in 144 (5·8%) out of 2454 cultures from 285 contacts and convalescents. These second examinations also frequently revealed the presence of isolated colonies of Hofmann's bacillus. Considerable importance ought therefore to be attached to second examinations, since no observations on the classes of persons just mentioned can be considered complete unless cultures, which at first show no diphtheria bacilli, are re-examined after a second period of incubation. Second examinations also frequently reveal the presence of Hofmann's bacilli in many healthy throats, in which their numbers are very small. Although absolutely accurate statistics of the number of times Hofmann's bacillus was discovered in the second and not in the first examination cannot be obtained from the writer's records, yet the unusually large percentage of normal persons recorded in Table D, p. 211, as harbouring these organisms is due in some measure to these secondary examinations. It was seldom found that they were missed on the first occasion, except in cases in which only one or two colonies were present amongst a copious growth of other organisms.

Staining of Fixed Preparations.

Loeffler's method is the one which has been most generally employed for staining the fixed preparations in whatever manner they have been made. This method when properly applied has been stated by many observers to give all the information which can be derived from the accessory methods of Neisser and others. The bodies of the bacilli show the characteristic irregularities in the protoplasm extremely well, and the metachromatic staining, in those which possess it, is well exhibited. Certain observers, therefore, state that for routine diagnostic purposes no other methods are necessary.

On the other hand, there are many workers who consider that Neisser's stain, or one of the other differential stains for the polar granules, is of great assistance, and almost invariably stain a second preparation from suspicious cultures in order to determine whether these granules are present or not.

Both these methods can be combined and much time saved by the method devised by *Cobbett.* This process consists of making on a cover-glass several parallel streaks from different colonies. The cover-glass is allowed to dry and then dropped film side down on a glass slide on which has been placed a drop of dilute Loeffler's methylene blue (1 : 5). The specimen is almost immediately firmly pressed down on filter paper with the cover-glass downwards, with the result that the excess of stain is forced from under the cover-glass and absorbed by the filter paper.

Immersion oil is then placed on the cover-glass and the preparation examined, mounted in a small quantity of the staining fluid. If the films are properly prepared[1] no bubbles are found under the cover-glass, and the organisms take up the stain and appear to lie in a clear fluid (Cobbett and Phillips, XII., 1896, p. 197). By this method the more concentrated portions of the protoplasm of the diphtheria bacilli are stained dark blue, the other portions light blue and the polar bodies are usually indicated.

Hofmann's bacilli stain darkly except for the median light band, and amongst these well stained specimens numerous almost unstained "shadows" are to be seen.

This method has very considerable advantages over the method of staining by Loeffler's blue and mounting in Canada balsam. In the first place much time is saved, the bacilli are not overstained nor are they liable to be distorted during manipulations or by the action of balsam. Comparative experiments have convinced the writer that in balsam the diphtheria bacilli appear smaller and shrunken, and are much less easily distinguishable from other organisms. Finally this procedure allows of 5 % acetic acid being run under the cover-glass as recommended by Cobbett (IX. 1901). A drop of the acid placed near the edge of the cover-glass generally runs quickly under the latter, and, if it does not do so, the withdrawing of a little fluid by placing a piece of filter paper at the opposite edge generally causes the acid to run in. If the preparation be watched during this process a wave of blue is seen to pass over it, and the bacilli are left almost decolorised except the polar bodies which stand out as dark black or blue dots. A single group of doubtful organisms can thus be readily examined for the presence of polar bodies without removing the eye from the microscope, a matter of great importance when the organisms are few, and it is doubtful whether they can again be obtained for making a preparation by Neisser's method.

---

[1] It has been stated that the cover-glass is apt to stick to the objective. This does not occur if large cover-glasses are used, and the excess of stain is removed by firm pressure.

Comparative experiments with this method and that described by Neisser show the results to be identical. The writer has used the method just described for the examination of several thousand cultures, and has found it extremely satisfactory in all respects.

Significance of Polar Bodies.

Although the presence of polar bodies is an aid to diagnosis yet, as has already been stated (p. 143), many diphtheria bacilli show none, and even when well developed their presence does not indicate that the organisms are virulent (p. 197). Further, several other species of bacilli resembling diphtheria bacilli also develop well-marked polar bodies within the usual limits of time in which cultures are examined. Hofmann's bacillus as a general rule in young cultures has no polar bodies, but in a few instances inconspicuous polar bodies few in number may be seen. In older cultures of this organism polar bodies are not infrequently seen. The polar bodies in these cases are generally, however, not so distinct, nor so dark, nor as uniform in size as those of the diphtheria bacillus. This fact is of importance when an examination of a culture 36 or more hours old is being made.

The Recognition of Diphtheria Bacilli.

The purpose for which the examination is made to some extent determines the time which can be devoted by the observer to the recognition of the bacilli. Bacteriological examinations may be undertaken for the following purposes:—(1) routine examinations for the diagnosis of diphtheria; (2) for the discovery of infected contacts; (3) for the release of convalescents and infected contacts from quarantine; (4) for the diagnosis of doubtful diphtheritic lesions in other situations than the throat and nose; (5) for proving the relationship of diphtheria and diphtheria-like bacilli to various morbid processes; (6) for obtaining statistics on the distribution of diphtheria bacilli amongst the normal population.

(1) In routine examinations undertaken for the purpose of giving opinions on the nature of cases clinically resembling diphtheria a diagnosis is expected in the majority of instances within 12 or 18 hours. The morphology and staining reactions together with the characters of the young colonies are the only features on which an opinion as to the nature of the organisms can be formed. The observer has therefore to rely on the results of his previous experience, since to be of any practical value the opinion of the bacteriologist cannot await the preparation of pure cultures and the injection of animals. Of the nature of bacilli which exhibit the typical characters of diphtheria bacilli there can be little doubt, but organisms will occasionally be met with which differ from the usual types in some particulars, and which have not been previously encountered by the observer. Under these conditions, wherever possible, a definite answer should be delayed, and the organisms subcultured so that their characters may be noted when not influenced by the presence of contaminating organisms. A longer delay will seldom be possible, and a diagnosis must be given on the data which have been obtained. It remains, however, for the bacteriologist to prove for his own benefit whether the opinion given was correct, by obtaining pure cultures and testing the principal cultural characters of the organism as well as its effect on animals. Unless such methods are adopted errors of judgment can only be perpetuated, and the opinion of the observer, however large his experience, will be of no value in doubtful cases. In the case of certain institutions and especially of private schools an opinion should be delayed when doubtful organisms are found in a primary case of supposed diphtheria. A

positive diagnosis of diphtheria not infrequently involves the owners in considerable pecuniary loss, and the patient can generally be isolated until certain characters of the bacilli have been tested. Unless the cultures are very greatly contaminated a pure culture can generally be obtained and its action on glucose broth tested within three or four days.

In the writer's experience bacilli which to some extent resemble diphtheria bacilli, but do not form acid in glucose broth, are not pathogenic to guinea pigs, and are therefore probably incapable of causing diphtheria in man. This experience is in complete agreement with that of Cobbett and many other observers. On the other hand bacilli which do form acid and grow in the typical manner of diphtheria bacilli in broth, and which are derived from persons suffering from lesions in any way resembling diphtheria, usually prove to be virulent diphtheria bacilli. In conclusion it may be said without hesitation "that when once one has become fully acquainted with the range of its variation it is fairly easy to recognise the diphtheria bacillus and distinguish it from all others," but "the eye cannot be sufficiently trained for this purpose unless the observer frequently tests the opinions he forms on morphological grounds by isolating his cultures, and testing them in various ways, including the injection of animals" (Cobbett, IV., 1901, p. 236).

(2) The examination of contacts should be carried out with the same precautions, and in their case it is almost always possible to delay a definite opinion, until at least the action of pure cultures of doubtful bacilli on glucose media has been determined. Here again much inconvenience and even pecuniary loss may be caused by hasty opinions, which are subsequently proved to be erroneous. The value of a further period of incubation for negative cultures and subsequent second examinations in these cases has already been pointed out.

(3) The examination of convalescent cases of diphtheria and of infected contacts for the purpose of deciding whether the diphtheria bacilli have disappeared, or not, is during the earlier stages usually an easy matter, as the organisms are frequently abundant. In the later stages, however, when the bacilli are beginning to disappear a negative verdict should not be given until the culture has been thoroughly examined after a further period of incubation, and doubtful bacilli, as in all other cases, should be carefully tested. As long ago as 1895 Ohlmacher (2. III. 95) pointed out that diphtheria bacilli may sometimes be missed in cultures from convalescents unless they are incubated for longer than the usual time.

(4) The examinations of discharges from the female genital organs, ears, ulcers, abscesses, etc., and of skin lesions[1], and also of milk and various other materials, for the presence of diphtheria bacilli are rendered difficult by the fact that in all these situations innocuous diphtheria-like bacilli are frequently present, which are in no way related to the disease. In such examinations, therefore, no final opinion should be given until the identity of the organisms has been thoroughly proved, however closely they resemble diphtheria bacilli.

(5) Mention must also be made of a class of investigation which aims at proving the association of diphtheria or diphtheria-like bacilli with certain morbid processes, not generally recognised as being related to diphtheria. It would seem scarcely necessary to point out that the bacilli should be tested in every possible way, including the effect of animal inoculations. Nevertheless many hasty generalisations, which if adopted would have the most far reaching consequences, have been based on morphology alone. To those who have any acquaintance with bacteriology the worthless nature of such observations is obvious, but they are apt to mislead a large number of persons who have no practical acquaintance with the subject, but are willing to place reliance on the conclusions of bacteriologists.

(6) Another class of observations to which attention has to be directed is that which deals with the presence or absence of diphtheria bacilli in the mouths of perfectly healthy persons, who have not recently had an opportunity of acquiring diphtheria bacilli by contact. It has already been pointed out (p. 189) that, if these observations are to form a basis for general arguments, all possible recent contacts must be excluded. Further, the animal inoculation test must be applied to all organisms which the observer considers to be diphtheria bacilli, since it has been shown that almost all the so-called diphtheria bacilli which have been found amongst these persons have been devoid of virulence. Whether the non-virulent diphtheria bacillus will ultimately be proved a distinct species from the virulent, as some observers think, or not, there is some evidence to show that the former is not capable of giving rise to diphtheria in man. Any arguments, based on the prevalence of diphtheria bacilli amongst the normal population and the transmission of the disease by this means, ought to take these facts into account. Many of the observers have, however, not tested the pathogenic properties of the bacilli they have found, or only have tested a small percentage of them,

---

[1] The characters of some of the organisms found in these situations are given in the following pages.

but in many instances they have assumed that all morphologically typical diphtheria bacilli must be potential carriers of the disease.

One of the latest and most extended investigations which have been carried out on the occurrence of diphtheria bacilli in well-persons is that of the Massachusetts Association of Boards of Health (1902). The several collaborators were requested to detail the various bacilli according to Wesbrook's types, and also to state in each case whether or not a positive diagnosis of the presence of diphtheria bacilli had been made. The different collaborators held divergent views as to which of the various types should be considered as true diphtheria bacilli, and in this investigation only a small proportion was tested for virulence. This report gives a good example of the unsatisfactory confusion to which a lack of thorough testing can lead.

In Providence (*Report*, footnote, p. 24), on the basis of the Committee's belief that A, C, and D of Wesbrook's types should be considered chiefly, or solely, important, there would be ·43 % of positives (*i.e.*, cultures in which diphtheria bacilli were present). If all granular, or barred forms, but not the solid forms, be included, as Prof. Gorham of Providence states, there would be 3 % of positives. If all be included there would be 25 %. The number actually reported positive (*i.e.*, diphtheria bacilli present) makes about 9 %.

In Washington the positives formed ·9 % on the Committee's standard, but 22 % were reported positive. In Boston on the Committee's standard 3·02 % were positive, but only 1 % was so reported.

The whole question of the recognition of diphtheria bacilli may be summed up in a few words.

In the practical diagnosis of diphtheria it must be assumed that organisms morphologically resembling diphtheria bacilli are true diphtheria bacilli, for the opinion of the bacteriologist to be of any practical value cannot await the preparation of pure cultures and the injection of animals; but if far reaching deductions are to be drawn from the observations the testing of the organisms found is essential, especially if the deductions are to be made the bases on which epidemics of diphtheria are to be combated by bacteriological means.

Apparently in consequence of omitting to verify their diagnoses by isolating and testing doubtful organisms, very divergent views are held even at the present day by various observers as to which of the various types of organisms should be considered dangerous. This unsatisfactory confusion renders many of the investigations untrustworthy, and can only lead to the discredit of the bacteriological diagnosis of diphtheria.

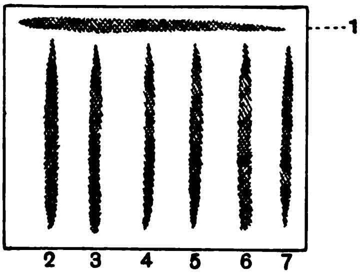
The diphtheroid organisms met with in cultures are either diphtheria bacilli (virulent or non-virulent) or they are not diphtheria bacilli. By means of cultural tests and animal experiments it is generally easy to distinguish between these groups; and even on morphology alone it is usually not difficult to give an accurate opinion, provided that the observer has systematically identified the organisms he has previously met with. It is therefore the duty of every bacteriologist who has to deal with this disease to make himself acquainted with the range in variation of the diphtheria bacillus and the organisms which resemble it, and to verify his diagnosis by every means in his power. By the adoption of these means alone can the opinions, which he gives on the examinations of his cultures, be of any value. It cannot be too often repeated that the examination merely of large numbers without proper controlling tests and without a knowledge of the origin of the cultures cannot be of service in the formation of trustworthy opinions.

Methods of obtaining Pure Cultures.

From the majority of original cultures it is easy to obtain pure cultures in one or two generations. The simplest method is to touch a suspected colony with the point of a sterilised platinum needle and immediately inoculate a tube of sterile salt solution (·7 %). In order to ascertain whether the type of bacillus, which it is desired to investigate has been sown in the salt solution, a smear is made from the needle on a cover-glass immediately after the former has been removed from the salt solution and before it has been sterilised. A minute drop from the salt solution tube is inoculated by means of a sterile platinum loop onto the surface of another serum tube. After incubation well separated colonies develop. Should the culture thus obtained be found by microscopic examination to contain colonies of other organisms besides those which are under investigation, the same procedure is repeated and a pure culture is obtained.

If the colonies on the original culture are very minute and the culture is not greatly crowded with contaminating colonies, further incubation adds to the growth of the separate colonies, and a pure culture can be obtained with greater ease. From very crowded cultures with minute colonies the first subculture is certain to be impure, but a pure culture can generally be obtained in two generations.

More difficulty is experienced when only one or two colonies of doubtful organisms are present amongst many other colonies which resemble them. In this case several salt solution tubes may be inoculated from difficult colonies, and numbered. Before sterilising the needle on each occasion a streak is made on a cover-glass. The streak corresponding to the first tube should run along one side of the cover-glass and the other streaks should be made in order at right angles to it, beginning on the left. There are then on one cover-glass several streaks from different colonies, corresponding to the salt solution tubes which have been sown from these colonies, arranged as shown in the following figure:



If the cover-glass is now stained and mounted it can readily be seen by noting the number of the streak, which shows the desired organisms, which salt solution tube must be used for subcultivation.

Various other methods, such as plating on agar, etc., may also be used for obtaining pure cultures.

The Distinguishing Cultural Characters of the Diphtheria Bacillus.

The principal cultural character, which distinguishes the diphtheria from the Hofmann's bacillus, is the formation of acid by the former in media containing glucose as opposed to the alkaline reaction produced by the latter. Abundant acid production also distinguishes the diphtheria from the xerosis bacillus, which produces, according to some observers, a slight amount of acid, according to others, none.

Certain other diphtheroid organisms also produce acid, but differ from the diphtheria bacillus in other respects, as in the appearance of their colonies and their rate of growth on various media.

Both the diphtheria and the Hofmann's bacillus produce an invisible or almost invisible growth on potato, while many of the diphtheria-like organisms produce abundant, and frequently coloured, growths.

The peculiar granular deposit together with the lack of clouding in broth cultures distinguishes the diphtheria bacillus from the Hofmann's bacillus, which produces more growth and some clouding, and from some of the other diphtheroid bacilli which produce very different growths. Neither the diphtheria bacillus nor the Hofmann's bacillus produces pigmented colonies on any media except serum, and even on this medium a yellowish coloration of the colonies is the only evidence of pigment production. Some diphtheria-like organisms liquefy gelatin.

So far as the writer's experience goes all organisms which produce an alkaline reaction to litmus in glucose broth are found to be non-pathogenic.

The Distinguishing Pathogenic Properties of the Diphtheria Bacillus.

The virulent diphtheria bacillus when inoculated in pure culture under the skin of the guinea-pig produces certain well-marked lesions, namely a small necrotic area at the site of injection, gelatinous subcutaneous oedema, intense congestion of the suprarenals and frequently clear yellow pleuritic effusion (p. 173). The simultaneous injection of antitoxin together with the culture protects the animal from the effects of the bacillus.

Less virulent diphtheria bacilli or smaller doses produce only oedema from which the animals recover, and these effects are also counteracted by antitoxin.

Experimental animals which recover either with or without the aid of antitoxin frequently suffer from paralysis.

Since some of the diphtheria-like organisms kill the inoculated animals by a septicaemic process (antitoxin either producing no effect or hastening death), autopsies on the experimental animals ought never to be omitted.

The Methods of recording the Morphological Appearances of Diphtheria Bacilli.

For recording the morphological appearances of diphtheroid bacilli met with in cultures Wesbrook's classification may be conveniently used. It must, however, be borne in mind that by this means a general idea of the morphological appearance of an organism alone is given, but no proof that it bears any relationship to the diphtheria bacillus. In most cases it is found that the majority of organisms composing a colony are of one type, but others belonging to one of the other types also occur. In recording the morphology of a pure culture or colony the general appearance conveyed to the eye is usually of more importance than a minute analysis of the slight variations from the general type, which may be found by diligent search. The method of growth of diphtheria and allied bacilli, and the different ages of the individuals comprising the colony, make it certain that a variety of individuals will be found. Nevertheless any pure culture has a characteristic general appearance, and most of the individuals belong to one type. Consequently in describing a culture it is the general character which it is important to convey.

Uncommon Diphtheritic Lesions, and the Diphtheria-like Bacilli found in Various Situations.

In the following pages are given a number of instances of diphtheritic lesions in unusual situations, and the characters of diphtheria-like organisms which have been found in these situations.

The mouth.

Diphtheritic lesions of the mouth are usually either confined to the neighbourhood of the tonsils, or are most marked there, and spread to other situations. Some of the more remarkable examples of diphtheritic lesions affecting other portions of the buccal cavity are quoted below:

Stomatitis. Councilman, Mallory, and Pearce (1900) observed general stomatitis due to diphtheria bacilli in five cases. *Tongue.* Membrane on the tongue due to virulent diphtheria bacilli has been noted by Wharton (1895). Trevelyan (1900), Thiercelin (1890), and Salmon (1904) have also observed membranous lesions on the tongue associated with diphtheria bacilli. In Salmon's case membranes were also present on the lips, palate, cheek, vulva, vagina, and rectum. Animal inoculations do not appear to have been made in these cases. *Lips.* Trevelyan (1900) recounts a case of diphtheria of the inner surfaces of the upper and lower lips. Long diphtheria bacilli were found, but their virulence was not determined. Flexner and Pease (1895) quote two remarkable examples of diphtheria of the lips and gums first noticed at autopsies. "The appearances of the membrane in one and the exudate in the other case were in no way typical of diphtheria, and the cases obtain an additional interest from the fact that during life the patients presented no symptoms referable to the presence of the Loeffler's bacillus. No other forms of diphtheria existed in the body so far as could be determined." In both cases the organisms were morphologically and culturally typical, but only caused a well-marked local reaction in guinea-pigs.

In the case of a patient suffering from diphtheria, who developed *herpes labialis* on the 9th day, Tez (1895) found virulent diphtheria bacilli in the vesicles. *Cancrum oris.* Several cases of cancrum oris associated with the presence of the diphtheria bacillus have been described. Freymouth and Petruschky (1898) observed two cases, Councilman, Mallory and Pearce (1900) two cases and Walsh (1903) eight cases. The first mentioned observers isolated the bacilli and found them to be in each case non-pathogenic for guinea-pigs in doses of 2 c.c.

PLATE XIV.

Fig. 1. Photograph of a smear preparation stained with Loeffler's methylene blue of *B. coryzae segmentosus*, grown on serum for 18 hours at 37° C. × 1000. (From Gordon, 1903, Plate VIII, fig. 9.) (See p. 361.)

Fig. 2. Photograph of a smear preparation stained with Loeffler's methylene blue of a chromogenic organism from the eye, grown on serum for 18 hours at 37° C. × 1000. (From Gordon, 1903, Plate VIII, fig. 7.) (See organism No. 22, p. 366.)

Fig. 3. Photograph of a smear preparation stained with carbol-fuchsin and Neisser's method of a streptothrix obtained from the throat of a diphtheria patient. One day agar culture grown at 37° C. × 1000. (From Gordon, 1903, Plate XI, fig. 17) (p. 357).

Fig. 4. Fusiform bacilli and spirilla in a smear preparation stained by Giemsa's method made directly onto a cover-glass from a case of Vincent's angina. Drawn with the aid of a camera lucida (Orig.). Zeiss $\frac{1}{12}$, Oc. 4.

Fig. 5. Photograph of a smear preparation stained with carbol-fuchsin of a pure culture of the fusiform bacillus grown five days anaerobically in dextrose-free broth. × 1200. (From Weaver and Tunnicliff, 1905, Plate XVI, fig. 1.)

Fig. 6. Photograph of a smear preparation stained with carbol-fuchsin of a pure culture of the fusiform bacillus grown for three days anaerobically on an ascites agar slant. × 1000. (From Weaver and Tunnicliff, 1905, Plate XVI, fig. 2.)

Diphtheria-like organisms from the throat.

A considerable number of organisms have been isolated from the throat which, although they resemble the diphtheria bacillus in

PLATE XIV

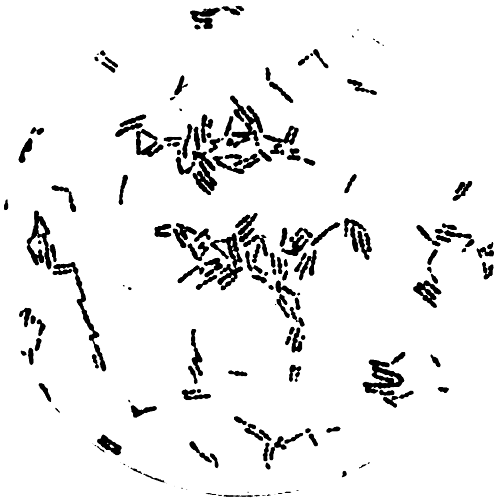


Fig. 1.

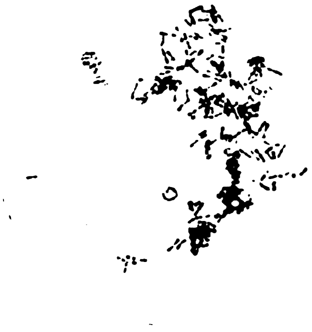


Fig. 2.

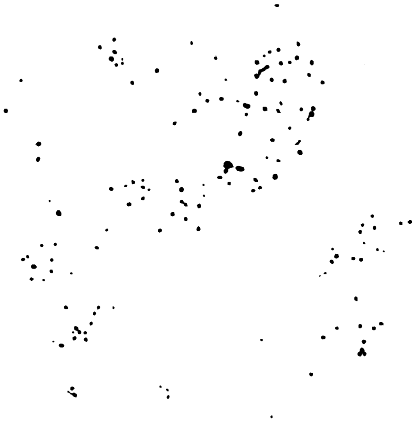


Fig. 3.

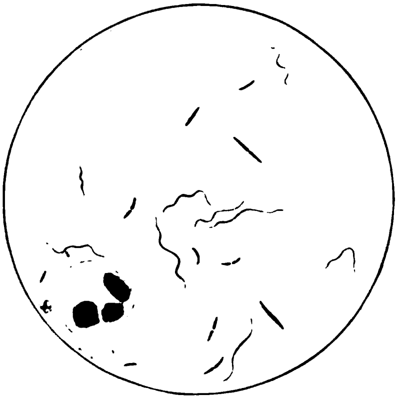


Fig. 4.

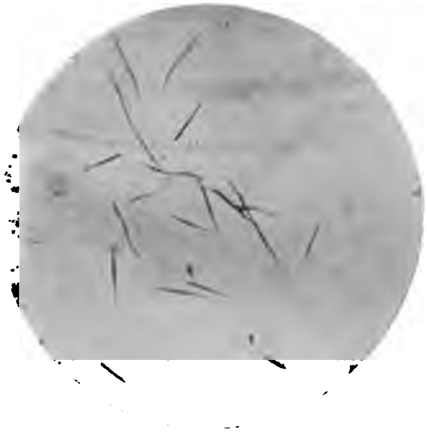


Fig. 5.

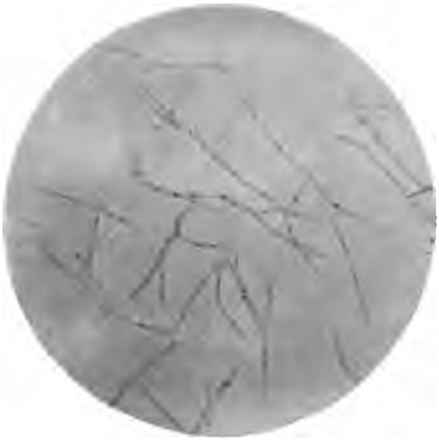


Fig. 6.

morphology, differ from it in certain of their characters. The true diphtheria bacillus is characterised by its:

(1) Macroscopic growth on *serum* and morphology, (2) power of producing acid in glucose broth, (3) invisible growth on potato, and (4) the lesions produced in inoculated guinea-pigs.

The diphtheroid organisms are here classified according to the number of points in which they differ from the diphtheria bacillus.

A. *Microorganisms resembling the diphtheria bacillus in all the main characters except virulence.*

Under this heading might be included the non-virulent diphtheria bacillus.

1.[1] *Bacillus maculatus.* (Plate XV, fig. 5.)

Origin. One colony found in a culture from the throat of a possible contact.

On serum in 24 hours the colonies are opaque white, but otherwise resemble those of the diphtheria bacillus. The organisms are longer and broader than diphtheria bacilli, but some short forms occur. The sides in some have slight bulgings at intervals. Numerous darkly stained segments cross the bacillus transversely in most of the organisms, but in a few there are oval segments. In some bacilli, especially in the later subcultures, long unstained intervals are seen. The organisms are non-motile, and retain Gram's stain very deeply. Each bacillus shows numerous polar bodies by Neisser's method; some of these are large and round, others elongated transversely across the bacillus, whilst others are very minute. These minute polar bodies are often very densely aggregated. The name indicates the remarkable spotted appearance seen when the organisms are stained by Neisser's method.

In the first culture the organisms lay in tangled masses of 10—50 individuals. On agar in 24 hours minute, round, transparent colonies are formed which subsequently grow very slowly. The organisms are about one-third the length of those found on serum cultures and of various shapes, from oval to bloated pear-shaped bodies. The polar bodies are few. On the surface of agar stab cultures an almost transparent film is formed, but in the depth medium-sized yellowish colonies grow. On gelatin after 10 days' growth the colonies are so minute as to be scarcely visible with a lens. Exceedingly minute colonies also form in the depth of gelatin stab cultures. On potato there is no visible growth. Broth remains clear, but a few large, discrete, yellow granules (0·5 cm. in diameter) are seen after 48 hours. The reaction of glucose broth becomes very faintly acid. Milk remains unchanged and no indol is formed. The organisms are non-pathogenic when injected either subcutaneously or intraperitoneally into guinea-pigs.

This organism differs from the diphtheria bacillus slightly in its morphology, its growth on gelatin, and in the very large size of the granules formed in broth, and the degree of acid formation in glucose broth.

---

[1] For the sake of reference the organisms described in the following pages are consecutively numbered.

B. *Organisms mainly differing from the diphtheria bacillus in their macroscopic growth on serum and in lack of virulence.*

Gordon (1903, p. 429) describes two species of bacilli of this kind.

2. After 18 hours' incubation a good growth was obtained on *serum*, but so coherent that to obtain a microscopical view of the individuals it was necessary to separate the growth by crushing between cover-glasses. Morphologically the bacilli are short and stout and resemble Hofmann's bacillus, retain Gram's stain and have well developed polar bodies at each end. On *agar* dry, white, circular, raised coherent colonies are formed. On *gelatin* there is no growth. *Broth* remains clear with coherent masses at the bottom. *Glucose broth* becomes acid. ***Milk*** is not changed. The organism is non-pathogenic.

3. After 18 hours' growth on *serum*, gray sharply outlined coherent colonies are formed. The organisms are curved and have swollen ends and occasional branching forms are encountered. There is however much variation in size and shape, some of the organisms being oval and some resembling mycelial threads. Loeffler's methylene blue stains them irregularly and they retain Gram's stain, but show no polar bodies by Neisser's method. There is no growth on *gelatin*. In *broth* coherent masses form at the bottom and *glucose broth* becomes acid. ***Milk*** becomes acid and by the 9th day is firmly clotted. Non-pathogenic.

C. *Microorganisms which resemble the diphtheria bacillus in all their main characters except virulence, but liquefy gelatin and cause clotting in milk.*

4. Gordon (1903, p. 431, No. 10).

Origin. Obtained from four patients suffering from diphtheria.

On *serum* after 18 hours moist raised gray colonies are produced. The organisms show great variations in size and shape, varying from oval to pear-shaped. Some very closely resemble diphtheria bacilli. Most stain evenly and deeply, but some show unstained portions and granules. Polar bodies are seen in many when stained by Neisser's method. They retain Gram's stain. On *agar* round, granular, raised colonies are formed which adhere to the medium and the organisms mostly resemble cocci, but some diphtheria-like forms occur. *Glucose broth* becomes acid. On *gelatin* there is a slight raised growth, and after 10 days' growth the medium begins to be liquefied. ***Milk*** becomes acid and on the 6th day is firmly clotted. Non-pathogenic.

D. *Microorganisms mainly differing from the diphtheria bacillus in their growth on potato and in their lack of virulence.*

5. *Bacillus diphtheroides citreus.* (Plate XV, fig. 3.) Graham-Smith (1904).

Origin. Obtained from the throats of five healthy children, two attending infected, and three non-infected schools.

On *serum* in 24 hours the colonies closely resemble those of the diphtheria bacillus, but are rather larger and more opaque. After three days' growth they become slightly yellowish. The organisms are fairly long and stain darkly, in shape resembling Hofmann's bacilli, but with little trace of a median band. The majority are slightly curved, and show small terminal polar bodies, though in some of the larger forms they are very large and distinct. They are non-motile, and retain Gram's stain. In 48 hours' subcultures a number of long forms with three or four well-marked segments and distinct polar bodies occur. On *agar* in 24 hours large, white, round, smooth, moist, dome-shaped colonies are formed. The organisms are the same in appearance as on serum, but the polar bodies are better marked. In agar stab cultures, a flat, thick, moist, white surface growth with indented edges is formed. The indentations are very evident after 48 hours' growth. Along the needle track the growth is well marked. No growth was obtained on *gelatin*. On *potato* in 24 hours a very extensive pale yellow moist growth occurs. The organisms are mostly short, but a number of markedly clubbed and segmented (up to six or eight segments) forms are found. All show large and distinct polar bodies. *Broth* after 48 hours is clear with white rather stringy deposit. In *glucose broth* the deposit is copious and occurs in the form of large white flocculent masses which tend to stick to the sides. The reaction is very acid. Non-pathogenic.

This organism differs slightly from the diphtheria bacillus in morphology though somewhat resembling the uniformly staining type (Plate VI, fig. 6), and also differs in its growth on broth and potato. It is from its marked growth on the latter that the name has been given. It resembles the organism next described very closely, except in its growth on gelatin, potato, broth and glucose broth. It may be the same organism as that described by Abbott (1891).

6. *Bacillus diphtheroides brevis.* (Plate XV, fig. 4.) Graham-Smith (1904).

Origin. Obtained from a large abscess cavity opening into the mouth.

The colonies on *serum* in 24 hours are small, smooth, and white, but not so well raised as those of the diphtheria bacillus. Subsequently they develop somewhat filmy edges. The organisms in shape resemble Hofmann's bacilli, but are slightly curved and clubbed and show segmentation. The segments stain darkly, but the intervening bands except the middle one are not very definitely marked. They are non-motile, retain Gram's stain, and show polar bodies by Neisser's method. In subsequent subcultures segmentation is a marked feature. On *agar* stroke cultures an abundant, white, soft, slimy growth is produced in 24 hours. The organisms are long, well curved, clubbed and segmented, and show good polar bodies resembling the diphtheria bacillus shown in Plate VI, fig. 4. Agar stab cultures show abundant white slimy surface growth which frequently becomes after further growth coarsely granular. A confluent, abundant growth occurs along the needle track with numerous projecting colonies. On *gelatin* in three days, medium-sized, round, smooth, dry-looking colonies with raised centres develop. On gelatin stab cultures a large, white, dry, granular surface growth is formed, and small round colonies develop in the needle track. On *potato* in 24 hours an extensive growth, thick, soft, and cream-coloured, is formed which gradually becomes granular and slightly yellowish. The organisms vary in appearance, some are short, but many long and well segmented with good polar bodies. In 48 hours *broth* becomes slightly cloudy and there is a fine whitish granular deposit. The reaction of *glucose broth* is extremely acid. *Milk* becomes partially coagulated in a few days and *indol* is produced. It is non-pathogenic.

This organism differs from the diphtheria bacillus in its growth on agar and potato, and somewhat in its morphology.

7. *Origin.* Obtained from the throat of a healthy child. (Graham-Smith, unpublished.)

After 18 hours' growth the colonies or *serum* are large, smooth, moist and yellow. The organisms are of medium length, slightly curved and have a central light band. In some the ends are oval or club-shaped, in others pointed. All are characterised by having an enormous polar body at each end. On *agar* minute, round, flat, delicate slightly granular, gray colonies are formed and the bacilli are segmented and exactly resemble diphtheria bacilli. *Broth* cultures show a thick adherent film and a yellow stringy deposit, and the fluid is slightly clouded. *Glucose broth* becomes acid. A good growth of small moist white colonies occurs on *gelatin*. After 24 hours there is a slightly raised yellowish growth on *potato* which in a few days becomes very abundant, dry and yellow. Non-pathogenic.

8. *Origin.* Obtained from a case of diphtheria.

On *serum* an abundant creamy growth occurs which later becomes yellow. After 18 hours' growth the organisms appear as short wedge-shaped and swollen rods, which do not show granules by Neisser's method. On *glycerine agar* and *potato* an abundant yellow growth occurs. Broth is made acid. Non-pathogenic. (Hamilton, 1904, No. 10.)

9. *Origin.* From the throat of a patient suffering from diphtheria.

On *serum* produces a moist colourless growth, on which medium the organisms appear as slender, barred and uniformly stained rods. They show no granules by Neisser's stain. On *glycerine agar* there is abundant growth and the agar turns brown. On *potato* an abundant gray shining growth is produced. *Broth* becomes cloudy and finally acid. Non-pathogenic. (Hamilton, 1904, No. 15.)

Eyre and Flashman (1904, p. 1109) isolated from the throat of an insane patient, a bacillus corresponding in all respects to an organism previously found by the former (1900) in milk, named "Diphtheroid No. 1." (See p. 329.)

E. *Organisms differing from the diphtheria bacillus in all their main characters except morphology.*

10. *Origin.* Obtained from the throat of a healthy child. (Graham-Smith, unpublished.)

After 24 hours' incubation the colonies on *serum* are still very small, but after 48 hours they become large and show a well raised, granular, irregular, opaque centre surrounded by a large, thin, granular zone with irregular margin. The bacilli are long and curved and occasionally clubbed and many show several bands. Terminal polar bodies are well marked. Although any single organism is indistinguishable from the diphtheria bacillus, masses are occasionally seen with the bacilli lying in parallel rows unlike diphtheria bacilli. On *agar* round, white, smooth, moist raised colonies are produced. There is no growth on *gelatin*. *Broth* cultures show a marked surface film of scale-like detached pieces, and a deposit of large flocculent granules. The fluid remains clear. In *glucose broth* no acid is formed. On *potato* there is a slight yellowish growth, and many of the organisms are markedly curved and show numerous segments. Non-pathogenic.

11. *Origin.* From the throat of a scarlet-fever patient.

On *serum* an abundant colourless growth is produced. After 18 hours' growth the organisms appear as slender, solid and long granular rods, and after further

DIPHTHERIA-LIKE BACILLI FROM SERUM CULTURES.

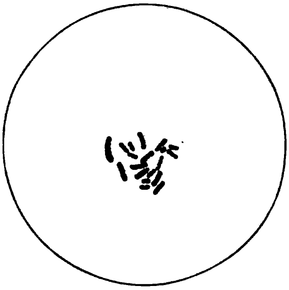


Fig. 1.

Hofmann's bacillus (Pseudo-diphtheria type). First culture 24 hours.

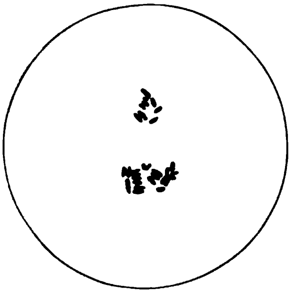


Fig. 2.

Typical Hofmann's bacillus. Subcultures from Pseudo-diphtheria type 24 hours.

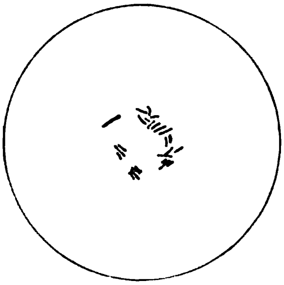


Fig. 3.

Bacillus diphtheroides citreus. First culture 24 hours.

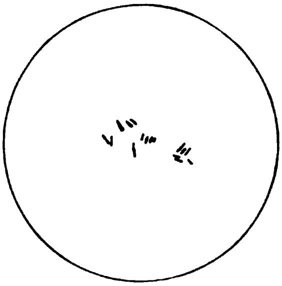


Fig. 4.

Bacillus diphtheroides brevis. First culture 24 hours.

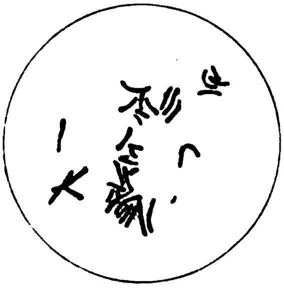


Fig. 5.

Bacillus maculatus. First culture 24 hours.

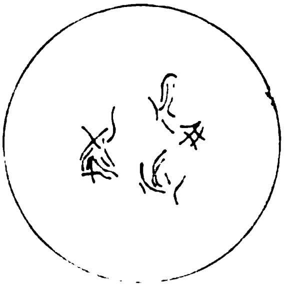


Fig. 6.

Bacillus diphtheroides liquefaciens. First culture 24 hours.

[From Graham-Smith, *Journ. of Hygiene*, Vol. IV. 1904.]

growth are like typical diphtheria bacilli. On *glycerine agar* and *potato* abundant colourless growth is produced. *Broth* becomes cloudy and remains neutral. Non-pathogenic. (Hamilton, 1904, No. 4.)

F. *Organisms differing from the diphtheria bacillus in most of their main characters except morphology, and capable of liquefying serum and gelatin.*

12. *Bacillus diphtheroides liquefaciens.* (Plate XV, fig. 6.) (Graham-Smith, 1904.)

Origin. Found in considerable numbers in the mouth of a patient suspected to be suffering from diphtheria.

On *serum* minute rounded colonies are formed in 24 hours, but in 48 hours they are medium-sized, round, slightly yellowish, dome-shaped and opaque. After 10 days' growth the colonies have sunk into slight pits, and the medium become partially liquefied after being kept 20—30 days at room temperature. The organisms are very long, and markedly curved, and lie in groups more or less parallel to one another. There is very little clubbing, and but slight signs of segmentation, but all show well marked terminal and other polar bodies. Some specimens remain as unstained shadows. They are motile, but the movements are slow, and they retain Gram's stain. These organisms bear a fairly close resemblance to the diphtheria bacilli shown in Plate VI, fig. 5. On *agar* stroke cultures in 24 hours a thick, moist, smooth, slightly yellow, abundant growth is formed. The appearance of the organisms is the same as on serum. On the surface of agar stab cultures an extensive moist smooth growth occurs, which occasionally in old cultures shows concentric markings. In the depth the colonies run together to form a continuous growth. Some of the discrete colonies at the edges are rounded, but have blunt projections. On *gelatin* very minute, almost transparent colonies are formed. The medium becomes liquefied round them in about 10 days. In a few days liquefaction is complete with a whitish-yellow mass lying in clear fluid. On gelatin stabs in 3 days the small yellowish surface growth is lying in a small cup-shaped area of liquefaction. In 11 days there is a deep funnel-shaped hollow with yellowish growth at the bottom and very minute colonies along the lower part of the needle track. On *potato* in 3 days a thin extensive white growth is formed, which in 6 days is very abundant and yellow. The organisms are of medium length, markedly curved, thin and stain uniformly. Many are clubbed and the polar bodies are very minute. *Broth* in 48 hours is slightly cloudy, with a large deposit of finely granular matter. In 48 hours the reaction of *glucose broth* is neutral or faintly alkaline. In 6—8 days *litmus milk* is decolorised and firmly clotted. No *gas* is produced, but much *indol* is formed and nitrates reduced. It is non-pathogenic.

G. *Chromogenic organisms.*

13. *Origin.* From the throats of two cases of scarlet fever.

On *serum* there is an abundant growth which liquefies the medium. The bacilli are short solid rods with a few small granules, but show no granules by Neisser's method. On *agar* an abundant green growth is formed and the agar turns purple. On *potato* a brownish growth is formed. *Broth* becomes cloudy and acid. Non-pathogenic. (Hamilton, 1904, Nos. 16 and 17.)

H. *Diphtheria-like bacilli virulent to animals.*

14. Davis (1899) isolated from the mouth in certain cases of scarlet fever a short diphtheria-like non-motile bacillus, which in doses of 2 c.c. injected subcutaneously produced a general septicaemia and peritonitis in guinea-pigs without oedema or congestion at the site of inoculation. Guinea-pigs which received antitoxin died more quickly than those which did not. The organism does not form a toxin. "Morphologically, it bears a striking resemblance to the diphtheria bacillus. It is polymorphic, sometimes short, rather evenly stained, while the individuals lie in parallel lines; at other times it is long, lies irregularly, and gives the broken stain typical of *B. diphtheriae*." It stains by Neisser's and Gram's methods. On *serum* and *broth* the growth is like that of the diphtheria bacillus. On *agar* it produced colonies with dark centres and uneven edges, and an acid reaction in *glucose broth*. A. Williams (1898) also seems to have isolated the same species from a patient who had suffered from diphtheria six weeks before.

15. Ruediger (1903) has recently described an organism which he calls a virulent pseudo-diphtheria bacillus.

Seven strains of this organism were isolated from seven fatal cases of scarlet fever with gangrenous tonsilitis. Diphtheria antitoxin had no influence on the patients, and in fact in two cases its injection appeared to hasten death. The bacillus is described as resembling the true diphtheria bacillus in morphology, causing "uniform turbidity in *broth*, a soft, moist, and whitish growth on *agar*, a hardy light brown growth on *potato*, and turning litmus *milk* white in 5—6 days." Apparently all strains showed polar bodies by Neisser's method, and were agglutinated in dilutions of 1 in 200 by the serum of a rabbit which had received three injections of 24 hour broth cultures of strain No. 5. "Guinea-pigs are not protected against this organism by anti-diphtheria serum. All seven strains are pathogenic for guinea-pigs after having been kept on agar for several months, when injected intraperitoneally in doses of 4 to 5 c.c." At the autopsies "the serous cavities contained a moderate quantity of fluid. The liver, spleen, and kidneys were markedly congested. The organisms could be isolated from the peritoneal cavity, heart's blood, and internal organs." A protective serum for guinea-pigs against injections of living cultures was obtained from a rabbit.

Hamilton (1904) continued Ruediger's investigations and obtained several other examples of this organism[1]. In testing for virulence she used intraperitoneal injections of broth cultures in doses equal to 1 $^{0}/_{0}$ of the body weight of the guinea-pigs. In further experiments the dose was diminished according to results. The animals died within 18 to 24 hours, and the following changes were found at the autopsy. "The peritoneum contained a large quantity of clear, reddish fluid, the liver and kidneys were much congested, the latter often dripping blood on section. The spleen was unchanged, and there was no change in the adrenals. The bacilli could be isolated from the peritoneal and pleural fluids, the blood, the urine, and all the organs, and from the subcutaneous tissue in a few cases. When a bacillus has proved virulent to guinea-pigs two further experiments were made with it. A dose of diphtheria antitoxin, sufficient to protect against a lethal dose of diphtheria bacilli, was given to one guinea-pig together with the ascertained lethal dose of the organism in question. At the same time a second guinea-pig was given a lethal dose of the organism, and also a sufficient quantity of the serum of a rabbit immunised against Ruediger's bacillus to protect against a lethal dose of the latter." "The experiments show that diphtheria antitoxin has no power to protect against these organisms and in some cases even seemed to hasten death and Ruediger's serum has no power to protect against diphtheria bacilli."

---

[1] On some occasions the cultures of these organisms appear only to have been obtained with difficulty. Hamilton describes her method of securing cultures in the following words: "It happened several times that serum cultures 24—48 hours old, from a throat the smears from which had shown the presence of suspicious bacilli, would fail to show anything but cocci, and the cultures would then be rejected. Happening to re-examine such a culture after seven days, I found it full of slender straight and curved rods with bipolar granules. After that the cultures were made on glycerin agar as well as on serum, and, in case bacilli were found on neither, a loopful of the serum culture was transferred to a tube of broth, and from this tube a loopful to the water of condensation at the bottom of a tube of glycerin agar, and the tube tipped to allow the water to run over the inclined surface of the agar. From such a culture colonies of bacilli could usually be obtained, and, in case of failure, the original serum culture was allowed to stand at room temperature for four or five days, and the procedure repeated. By this means it was found possible to isolate bacilli in all cases in which the original smears had shown their presence."

Apparently at least two distinct species of organisms whose pathogenic properties are neutralised by Ruediger's serum were isolated, one from an ear discharge (see No. 29).

Of the first species seven examples were obtained from various lesions of the mouth some of which were diagnosed clinically as diphtheria. After 18 hours' growth on serum five are described as short, and two as like typical diphtheria bacilli. Four showed polar granules by Neisser's method. Most of them gave abundant growths on all media, but in the case of one the growths were scanty. All except one produced acid in broth, and in most the medium remained clear. In all cases animals were protected by Ruediger's serum, but not by diphtheria antitoxin.

Hamilton and Horton (1906) made further investigations on this group of organisms. They found that all their strains of typical diphtheria bacilli haemolysed rabbits' corpuscles, while all their typical pseudo-diphtheria bacilli did not. Only two out of 18 strains of virulent diphtheria-like bacilli, whose effects were not neutralised by antitoxin, haemolysed the corpuscles. Their most interesting observations are on the specific bacteriolysins produced in the blood of rabbits immunised against these bacilli. One of these strains was injected subcutaneously into rabbits at 7 day intervals beginning with a dose of 1 c.c., and finally reaching 12 c.c. The method of testing the bactericidal action was as follows—five tubes were used:

No. 1 contained 1 c.c. of plain broth.
No. 2 „ ·8 c.c. of broth and ·2 c.c. of normal serum.
No. 3 „ ·9 c.c. „ ·1 c.c. „
No. 4 „ ·8 c.c. „ ·2 c.c. of immune serum.
No. 5 „ ·9 c.c. „ ·1 c.c. „

After inoculation with the same quantity of culture these tubes were incubated and plates poured after 4, 6, 20 and 24 hours.

The following table, illustrating one of their experiments, shows that the serum of the immunised animals exerted a powerful bactericidal action on these races of bacilli, but had no effect on diphtheria or Hofmann's bacilli.

Table showing the effect of normal rabbit serum and the serum of rabbits immunised with virulent pseudo-diphtheria bacilli upon these organisms, diphtheria bacilli and Hofmann's bacilli.

	Normal rabbit serum				Immune rabbit serum							
	Serum 0·2 Broth 0·8		Serum 0·1 Broth 0·9		Serum 0·2 Broth 0·8		Serum 0·1 Broth 0·9		Serum 0·05 Broth 0·95		Control Broth 1 c.c.	
Bacilli	6 hrs.	24 hrs.	6 hrs.	24 hrs.	6 hrs.	24 hrs.	6 hrs.	24 hrs.	6 hrs.	24 hrs.	6 hrs.	24 hrs.
[1] Bacillus No. 1	6000	∞	6000	∞	0	1	27	35	50	496	∞	∞
,, No. 2	4224	∞	5000	∞	24	1	34	6	26	0	4000	∞
,, No. 3	400	7000	200	7100	25	700	8	600	30	330	560	6000
,, No. 4	2000	∞	2510	∞	0	0	200	0	–	–	8000	∞
Diphtheria bacillus 1	23	∞	21	∞	10	∞	37	∞	–	–	17	∞
,, ,, 2	570	3223	110	1616	800	3440	750	3880	850	4160	100	440
Diphtheria-like bacillus	1760	∞	–	–	1840	∞	–	–	–	–	1600	∞
[2] Hofmann's bacilli ? 1	560	–	600	840	720	440	2000	1000	–	–	4500	10000
2	600	∞	400	∞	350	∞	350	∞	480	∞	8000	∞
3	800	∞	8000	∞	8000	∞	8000	∞	8000	∞	750	∞
4	1440	∞	880	∞	1280	∞	720	∞	–	–	2000	∞

They observed that the bactericidal substance was very stable resisting the action of age, light, drying and heat (80—90° C.) to an unusual degree. The active principle is removed by filtration through porcelain. They also note that the immune serum contains an agglutinin, and that phagocytosis plays an important rôle in the protection of guinea-pigs against these bacilli.

As the result of their experiments they conclude that "the bacilli described form a distinct group from *B. diphtheriae* on the one hand, and other pseudo-diphtheria bacilli on the other. The distinction lies in the fact that goats and rabbits, immunised against one member of this group, yield serum which is bactericidal to other members of the group, but not to *B. diphtheriae* or other pseudo-diphtheria bacilli, and which contains opsonin specific for members of this group."

16. Hamilton (1904) also obtained another species of virulent diphtheria-like bacillus from one case diagnosed as diphtheria and two cases of measles. This organism killed guinea-pigs in the same manner as those just described, but the animals could be protected either by diphtheria antitoxin or Ruediger's serum.

After 18 hours' growth most of the organisms are short, but long and barred forms occur in some cultures. No granules are shown by Neisser's method. They produce abundant white or yellowish growths on all media, and *gas* in glucose agar. *Broth* is made cloudy and in some cases becomes acid.

---

[1] Bacilli Nos. 1 and 2 were isolated by Ruediger from the throats of scarlet-fever patients, No. 3 by Hamilton from the throat of a patient suffering from laryngitis, and No. 4 from the urine of a scarlet-fever patient.

[2] These organisms are described as "ordinary pseudo-diphtheria bacilli," but are not otherwise defined. They were derived from cases of otitis media, conjunctivitis, and two cases of scarlatinal sore throat.

I. *Streptothrices resembling diphtheria bacilli.*

17. Gordon (1903, No. 10) (Plate XIV, fig. 3) describes a streptothrix whose broken fragments closely resemble diphtheria bacilli, being slightly curved, showing polar granules, and retaining Gram's stain. This organism does not grow on *serum* but produces a good growth of raised, granular, well defined colonies on *agar*, which adhere to the medium. On *gelatin* a gray, slightly raised growth is produced which becomes wrinkled transversely, and begins to liquefy the medium on the 14th day. A faint turbidity and a slight sediment are produced in *broth*, and *glucose broth* is made acid. *Milk* is not altered. Non-pathogenic.

18. This organism was found very abundantly in the throat of a child suffering from a slight sore throat, and was at first mistaken for a diphtheria bacillus by an experienced bacteriologist. (Graham-Smith, unpublished.) The majority of forms met with in microscopic preparations are almost indistinguishable from diphtheria bacilli, being curved, slightly clubbed, and segmented and show well marked polar bodies. Small masses are, however, occasionally seen in which long thread-like forms are encountered, many times the length of the bacillary forms. These threads are arranged in interlaced masses, and each thread shows numerous polar bodies at intervals corresponding to the length of the bacillary forms. Branching specimens are occasionally seen. On *agar* the majority of specimens are exactly like diphtheria bacilli, but long thread-like forms are commoner than on serum. On *potato* most of the organisms are short, stain poorly, and are much swollen at one or both ends. Long filaments with swollen ends are occasionally encountered. Growth on *Loeffler's medium* or Cobbett's modification of Lorrain-Smith's medium is poor, but the organism grows well on solidified *calf's serum*. The colonies are just visible after 24 hours' incubation, and after three days' growth have attained the size of small diphtheria colonies. The smaller colonies are round but the large ones are somewhat irregular in outline, and the surface in all is slightly wrinkled and granular. The colonies adhere to the surface and can seldom be removed without taking up some of the medium. On *agar* after three days' incubation the colonies are white, granular, dry looking and raised above the surface. Their edges are extremely irregular and deeply indented, and the growth adheres firmly to the surface. No growth occurs on *gelatin*. *Broth* remains clear without any surface film, and a coarse granular deposit forms on the sides and bottom. A small amount of acid is formed in *glucose broth*. On *potato* the growth is invisible. The organism is non-pathogenic to guinea-pigs when injected in large doses either subcutaneously or intraperitoneally.

The bacillary forms of *Streptococcus scarlatinae* may so closely resemble diphtheria bacilli as to cause difficulty in diagnosis. (Gordon, 1900–1, Case VII. p. 365.)

Diphtheria of the nose.

That diphtheria may affect the nose has been well known for a long time. The disease may be confined to the nose, or the throat and nose may be attacked at the same time. It is also well recognised that nasal diphtheria may vary greatly in the severity of its manifestations, not infrequently being of so mild a nature as to be mistaken for a mild catarrh. Several instances of cases of this nature giving rise to severe faucial diphtheria have already been quoted (p. 310), and further statistics need scarcely be given. There are, however, some manifestations of nasal diphtheria which require special mention, namely (*a*) chronic fibrinous rhinitis, and some forms of (*b*) atrophic and (*c*) external rhinitis.

(*a*) *Fibrinous rhinitis.*

The name chronic membranous or fibrinous rhinitis has been applied to a certain class of cases of diphtheria in which the disease principally or solely affects the nasal mucous membrane, and is attended by little or no constitutional disturbance. Some writers have claimed that virulent diphtheria is seldom contracted from contact with a case of fibrinous rhinitis, but that on the other hand a case of fibrinous rhinitis has not infrequently been observed to give rise to another of the same kind.

Lieven (1891) indeed reported a case from which he obtained an organism that when introduced into the noses of other children by means of tampons caused a similar condition in them. Some observations on this point have already been quoted (p. 311). As it has been suggested that the diphtheria bacillus concerned in these cases may have a lower degree of virulence than usual, the observations of those workers who have investigated this question have been brought together in the following table. This table, however, in which all those bacilli which killed the inoculated guinea-pigs within five days have been considered as virulent, shows that decrease in virulence is not of common occurrence in this form of the disease, and consequently all cases of fibrinous rhinitis must be considered to be capable of communicating diphtheritic infection.

Lack (1899, p. 13) made the following experiment with a bacillus derived from a case of fibrinous rhinitis. "A large rabbit was tracheotomised and the tracheal mucous membrane rubbed with a pure culture of the bacillus. The animal died on the third day. The whole neck and a portion of the thorax was extremely oedematous with small scattered haemorrhages, and there was a membranous exudation lining the whole trachea and extending down to the bronchi." He further observed that powerful toxins were produced.

Observer	No. of cases investigated	Virulent diphtheria bacilli	Attenuated diphtheria bacilli
Pluder (1896)	6	3	3 ?
Lack (1898)	23	23	0
Neumann (1902)	7	6	1
Ravenel (1895)	7	6	1
Gerber and Podack (1895)	5	5	0
Stamm (1891)	4	4	0
Pugh (1902)	4	4	0
Townsend (quoted by Welch, 1894)	4	4	0
Wolff (1899)	4	4	0
Park (1892)	4	2	2
Morf (1899)	3	3	0
Abbott (1893)	3	2	1
Baginsky (1892)	2	2	0
Concetti (1892)	2	2	0
Dowson (1895)	2	2	0
Cobbett (1901)	1	1	0
Reichenbach (1900)	1	1	0
Abel (1894)	1	1	0
Möller (1902)	1	1	0
	84	76 (90·4 %)	8 (9·5 %)

In consequence of the common occurrence of diphtheria bacilli in fibrinous rhinitis all such cases must be considered to be diphtheritic until the contrary has been proved. Nevertheless all membranous conditions of the nose are not due to the presence of diphtheria bacilli, since in a certain proportion this organism cannot be demonstrated. Gerber (1905) in an investigation of 40 cases only found diphtheria bacilli in 29 (72·5 %), and Meyer (1896) found them in 13 (59 %) out of 22 cases. The latter observer could find no clinical difference between cases due to diphtheria bacilli and cases due to other organisms. Ravenel (1895) in his extensive investigations had previously arrived at the same conclusion. Some of the latter cases are apparently caused by staphylococci or streptococci, and a few by pneumococci (Möller, 1902, Abel, 1892).

(b) *Atrophic rhinitis.*

Symes (1903) has recently observed diphtheria bacilli in 20 (87 %) out of 23 cases of atrophic rhinitis; 17 being described as long, and three as short diphtheria bacilli. Morphology in culture was principally relied on. The ages of his patients varied between 9 and 57, and the mean duration of the symptoms was seven years. In one case the condition had been present for 45 years. He examined also a series of noses of healthy children and adults, but found in them no long diphtheria bacilli. A second control series of noses consisting of cases of ozaena, congenital and acquired syphilis, rhinitis sicca, and lesions other than atrophic rhinitis showed no diphtheria bacilli. Two out of the 17 long diphtheria bacilli found were tested for virulence, and both were found to be virulent. The author does not mention the reaction of the organisms in glucose media, and appears not to distinguish sharply between the diphtheria and Hofmann's bacillus, saying that among the normal noses in 58 % "a short diphtheria-like or pseudo-diphtheria type of bacillus was present in the nose."

(c) *External rhinitis.*

Todd (1898) observed 51 (14 %) cases of rhinitis subsequent to scarlet fever amongst 365 patients at the London Fever Hospital. The early symptoms are redness at the margin of one or both nostrils. Later the redness increases and the surface becomes raw and granular, and crusts are formed on the surface, which may remain from one to five weeks. Pustules were frequently formed on other portions of the body from contact with the discharge. In all 51 cases diphtheria bacilli were found together with staphylococci. None of these cases developed faucial diphtheria although three cases showed diphtheria bacilli in the throat. Pure cultures of the bacilli produced acid from glucose and behaved in all ways like diphtheria bacilli. Out of seven strains injected into guinea-pigs in doses of 1 c.c. six caused death within 60 hours, and one on the ninth day. Two strains injected in doses of ·2 and ·1 c.c. respectively did not kill the animals. Two control animals inoculated with virulent cultures together with antitoxin were not affected. Diphtheria bacilli were also found in the secondary pustules. The author differentiates between the diphtheria and Hofmann's bacillus.

Williams (1901) also calls attention to the occurrence of diphtheria and diphtheria-like bacilli in both clear and thick nasal discharges associated with scarlet fever. Out of 141 cases of post-scarlatinal rhinorrhoea examined, 57 showed bacilli morphologically resembling diphtheria bacilli. Of the 17 strains which he isolated and tested 11 formed acid in sugar broth, but eight of these 11 were totally non-pathogenic while the other three killed the inoculated animals within 24 hours. Control animals treated with antitoxin lived. Six produced no growth in sugar broth. Of these one was pathogenic and five non-pathogenic. Symes (1895) in a case of similar nature of seven weeks duration also found virulent diphtheria bacilli. Marsden (1901) observed diphtheria bacilli in the nasal discharges of 10 children with slight post-scarlatinal rhinitis. No virulence tests are reported.

The formation of membrane after operations on the nose and tonsils or after the application of the cautery appears to be not very uncommon. Observers, who have investigated these conditions, have seldom noticed the presence of diphtheria bacilli.

Diphtheria-like bacilli from the nose.

Cautley (1896) has described an organism which he has named the *Bacillus coryzae segmentosus*, obtained from the nasal secretion of seven out of eight cases of "influenza cold." Gordon (1903) and Benham (1906) have also isolated this organism and supplemented Cautley's account of it. According to the latter observer this organism is very frequently present in nasal catarrh. He found it in 25 out of 27 cases investigated. Prosser White (unpublished observation quoted by Benham) found it in 17 out of 21 cases. The writer has also observed this organism in nasal secretions and the following account is partly based on these observations:

19. *Bacillus coryzae segmentosus*, Cautley. (Plate XIV, fig. 1.)

On *serum* grows more slowly than the diphtheria bacillus, though the colonies are very similar in appearance. Organisms in 24 hours resemble Hofmann's bacilli with light median bands, but are longer. A considerable number of long forms resembling uniformly stained diphtheria bacilli (Plate XIV, fig. 1) are also found. They are non-motile, retain Gram's stain, and show small terminal polar bodies in most specimens. In subcultures many long segmented forms are seen, with two to four well stained segments, as well as shorter Hofmann-like forms. On *agar* in 48 hours round, smooth, white, raised colonies are formed. The organisms are long, well segmented, curved and clubbed, and but few short forms occur. Polar bodies are only found in a few. They resemble closely the diphtheria bacillus shown in Plate VI, fig. 3. In agar stab cultures in 24 hours a small, white, round, smooth surface growth is formed, which is very extensive after 48 hours. There is well marked growth in the depth. On *gelatin* at 22° C. small, round, white colonies are produced. On *potato* an almost invisible thin whitish growth is formed. *Broth* remains clear, and a *stringy* white deposit is found at the bottom of the tube. *Glucose* broth in 48 hours is very faintly acid. Benham found that it also produced acid from lactose, maltose and saccharose. According to the writer's observations (1906) acid is produced from glucose, galactose and laevulose, but not from maltose, dextrin, glycerine, lactose, mannite or saccharose after 10 days' growth in the serum medium of Hiss. *Milk* in some cases is made acid and clotted. Non-pathogenic.

20. De Simoni (1898) isolated from the nasal secretion of a case of ozaena, a diphtheria-like bacillus 2—4 μ in length. The organism is generally straight, but is sometimes slightly curved. The ends are rounded and one is generally thicker than the other, and the organism is distinctly segmented. Colonies on *agar* resemble those of the diphtheria bacillus. *Broth* becomes clouded and a slight surface growth and granular deposit are formed, and the medium becomes acid. The organism grows well on *serum*, forming large moist white colonies which coalesce, but no growth occurs on *gelatin*. *Milk* is not coagulated and on *potato* growth occurs as a delicate moist broad layer. In *milk* and on *potato* rounded *spores* are formed in the middle of the bacilli. The organism is non-pathogenic. This author (1899) has also described two other species of bacilli from the nose, which, however, do not resemble the diphtheria bacillus very closely in morphology.

Diphtheria of the eye.

Some observers consider diphtheria of the conjunctiva to be a fairly common disease, especially amongst the children in large towns. Burton (1899) and Stephenson (1900) both consider that about 2 % of all cases of ophthalmia in children treated in the Children's Hospitals are diphtheritic, and Gonin (1899) found diphtheria bacilli in seven out of 365 cases of conjunctivitis which he examined bacteriologically. On the other hand many other writers think that diphtheria of the conjunctiva is very rare. Pollock (1905), for example, states that "true diphtheria of the conjunctiva is a very rare condition in this country." The presence of virulent diphtheria bacilli in cases of conjunctivitis has been proved by several investigators, but their records are not sufficiently numerous to base statistics of the incidence of the disease on them. On the other hand many instances have been recorded of the finding of diphtheria bacilli in cases of conjunctivitis without sufficient proof of the identity of the organisms. Inoculation experiments are especially necessary in establishing the identity of diphtheria-like bacilli derived from the conjunctiva owing to the frequent occurrence in this situation of the Xerosis bacillus, which in morphology closely resembles the diphtheria bacillus.

Jessop (1895) was the first observer in England to record the occurrence of virulent diphtheria bacilli in a case of membranous conjunctivitis. He subsequently (1902) proved the presence of virulent bacilli in three other cases. Morax (1895) has recorded virulent diphtheria bacilli in four cases, Uhthoff (1901) in two, and Gordon (1903), Griffith (1901), Eyre (1897), and Guder (1895) in one each. Pilcher (1897) observed diphtheria bacilli in 10 cases, and was able to produce diphtheria of the injured conjunctiva in rabbits and

DIPHTHERIA-LIKE BACILLI FROM SERUM CULTURES.

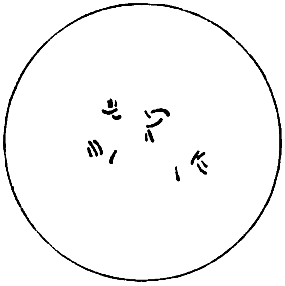


Fig. 1.
Bacillus xerosis.
First culture 30 hours.

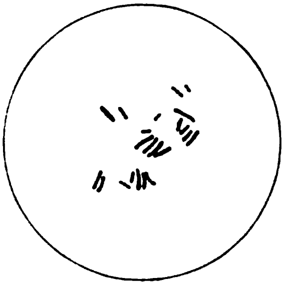


Fig. 2.
Bacillus xerosis canis.
First culture 48 hours.

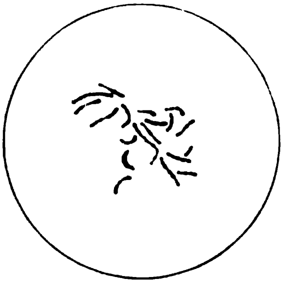


Fig. 3.
Bacillus auris.
First culture 48 hours.

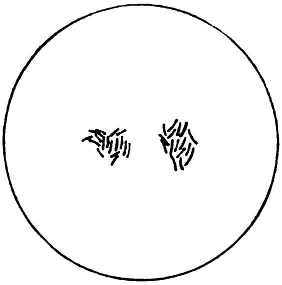


Fig. 4.
Bacillus ceruminis.
First culture 30 hours.

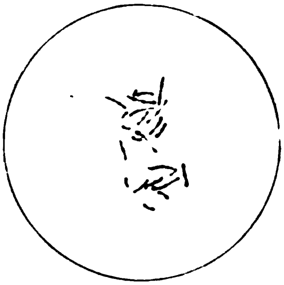


Fig. 5.
Bacillus diphtheroides gallinarum.
First culture 24 hours.

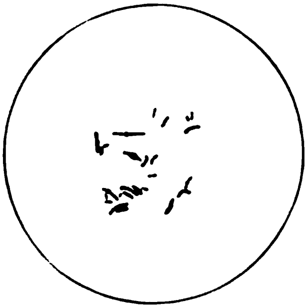


Fig. 6.
Bacillus No. 45, p. 381.

[From Graham-Smith, *Journ. of Hygiene*, Vol. IV. 1904.]

guinea-pigs, with the bacilli derived from some of these. On the other hand Kasatan (1903), Schanz (1897), Moritz (1895) and others have isolated acid-forming but non-virulent diphtheria-like bacilli from similar cases.

Diphtheria-like bacilli from the eye.

21. *The Xerosis bacillus.* (Plate XVI, fig. 1 and Plate IX, figs. 7 and 8.)

The term Xerosis bacillus has been used not only to denote the organism described below which is found both in the healthy and diseased conjunctiva, but has been applied also by some writers to the non-virulent, but otherwise typical diphtheria bacillus. This fact has led to some confusion in the writings on the subject.

As in the case of the Hofmann's bacillus opinions differ as to the relationship existing between the Xerosis bacillus and the diphtheria bacillus. While some hold that the two organisms belong to distinct species, others consider that the Xerosis bacillus is merely an attenuated diphtheria bacillus. Some have asserted that the Xerosis bacillus is capable of giving rise to various lesions of the conjunctiva, and others have denied that it possesses any pathogenic power.

In the following pages the characters and distribution of the Xerosis bacillus are first considered, later evidence for and against its pathogenic powers, and finally its relationship to the diphtheria bacillus.

The Xerosis bacillus on young *serum* cultures closely resembles in morphology the diphtheria bacillus. The bacilli are long, usually curved, and definitely broader at one or both ends than in the middle, and the majority show well marked segments, often very regularly arranged. Preparations made from cultures show the bacilli arranged in the same way as diphtheria bacilli, and Hill has pointed out that they belong to his "snapping" group and undergo the same post-fission movements as diphtheria bacilli. Denny (1903) has studied their morphology after various periods of growth, and remarks that after 8—12 hours' incubation the organisms stain uniformly and resemble Hofmann's bacilli. After 15 hours they are longer and show signs of segmentation, while after 24 hours they are still longer and the segmentation is more marked. After 36 hours segmentation is very well marked and the organisms are yet longer. While some observers state that they show polar granules by Neisser's method others state that they do not. This is probably due to the fact that polar bodies are not commonly found in many individuals in the original cultures, but are more common in subcultures. They are non-motile and retain Gram's stain. All observers agree that they grow more slowly on serum than do diphtheria bacilli. After 24 hours' incubation the colonies are still very minute, and even after 48 hours are smaller than those of young diphtheria bacilli. At this time the colonies are rounded, rather flattened, and have a dry, scaly appearance, and a gray colour. They further differ from the colonies of the diphtheria bacillus in adhering firmly to the surface of the medium. The edges of the colonies are uneven and the irregularity becomes more marked after further growth.

On *agar* minute, raised, round, smooth, almost transparent colonies make their appearance in 48 hours, which adhere closely to the medium. In *agar stabs* the surface growth is small and almost transparent, and very minute colonies are seen along the line of puncture. On this medium the organisms are broader than on serum, well segmented, but show no polar bodies. On *gelatin* a few strains only show any growth as very minute, round and transparent colonies. On *potato* growth occurs as an invisible film. *Broth* remains clear and growth occurs in the form of large or small granules which are found at the bottom of the tube. There is no surface growth. According to most observers there is no acid production in *glucose broth*. Knapp (1904) studied the action of 10 strains of this organism on 1 % solutions of various sugars in the serum medium of Hiss, and stated that they produced acid with dextrose, mannite, and saccharose, but not with dextrin.

Benham (1906) on the other hand states that it does not produce acid with glucose, lactose, saccharose or maltose.

According to the majority of observers it is entirely non-pathogenic to animals, but a few have stated that subcutaneous injection gives rise to a slight oedema. The Xerosis bacillus therefore differs from the diphtheria bacillus in the rate of growth, and in the appearance of its colonies on serum. It also differs in producing little or no acid in *glucose* broth, and in its entire lack of virulence.

The Xerosis bacillus was first discovered by Kuschbert and Neisser (1884) in Xerosis of the conjunctiva, and received its name from that disease. Subsequent observers have frequently found it in this disease. Stephenson (1898) examined clinically 6209 London school children and found that 1·87 % were suffering from Xerosis, and remarks that he has never failed to find the Xerosis bacillus in this condition. It has also been recorded in conjunctivitis of every description. Pollock (1905) observed "that colonies of the Xerosis bacillus were almost invariably present in culture tubes taken from muco-purulent conjunctivitis," and Griffith (1901) found it in 17 out of 20 cases of follicular conjunctivitis and in six out of 12 cases of slight conjunctivitis. It has also been recorded by various observers in true and chronic trachoma and several other conditions. Deyl (1893) isolated it in 15 cases of chalazion formation and considered it the cause of the disease, and stated that he had produced such formations in the eyes of animals by inoculation of the bacillus.

Although Franke (1893), Eyre and others have failed to find this organism in the normal conjunctiva there is ample evidence from the experiments of recent observers that it is frequently present.

Griffith (1901) found it very frequently, stating that from one loop passed over the conjunctiva 30 to 100 colonies were commonly obtained on serum cultures and once over 200 colonies grew. He concludes by observing that "the organism known as the Xerosis bacillus is the most common inhabitant of the conjunctival sac." Coppez (1899) states that it is an organism commonly found in the secretions of the eye, and

Lawson (1898) considers it to be "the most common and most universal inhabitant of the conjunctival sac." Heimersdorff (1898), Stephenson (1898), Fraenkel (1897) and Uhthoff (1896) also found it very frequently. The relative frequency of the occurrence of the Xerosis bacillus in the normal conjunctiva has been the subject of very few observations. Griffith (1901) in 210 examinations found it 120 (57·1 %) times, Lawson (1899) in 200 examinations 118 (59 %) times, and McKee (1906) in 140 examinations 42 (30 %) times. The writer has observed it in almost all the normal eyes he has examined.

The experimental evidence, as far as it goes, is entirely against the pathogenicity of this organism both towards men and the lower animals.

Piltz (1890), Braunschweig (1890), Fraenkel and Franke (1887), Lachowiez and others have inoculated the organism into the human conjunctiva without setting up any disease.

Stephenson (1898) made three sets of experiments on the human eye: "First, attempts to set up Xerosis in the second eye of a patient with one eye already affected, morsels of greasy material from the conjunctiva being used for the purpose; second, attempts to produce Xerosis in the eye of a second person by conveying to it some of the frothy material obtained from another subject; and third, attempts to set up the condition by inoculating healthy conjunctivae with pure cultures of Xerosis bacilli." All these experiments were without result. Many attempts have been made by numerous investigators to produce lesions in the eyes of animals with pure cultures of this organism, obtained from the normal and also from the inflamed eye, but all have failed. The methods employed have been inoculation onto the surface and under the conjunctiva and into the anterior chamber of the eye in dogs, rabbits, guinea-pigs and mice. Intravenous, intraperitoneal and subcutaneous inoculations even in large doses also produce little, if any, effect on the ordinary laboratory animals. The exceptional observation of Deyl (1893) has already been quoted (p. 364).

Owing to its general resemblance both in morphology and culture to the diphtheria bacillus, the Xerosis bacillus has been regarded by some observers as being a diphtheria bacillus which has lost its virulence, and it has even been suggested that the Xerosis bacillus may after passing down the ducts into the buccal cavity become under unknown conditions a true virulent diphtheria bacillus—Schanz (1894).

No experimental proof, however, of the relationship of the Xerosis to the diphtheria bacillus has been brought forward, and those[1] who have specially studied the former organism within recent years have almost without exception taken the view that the two organisms are distinct species.

Other diphtheria-like organisms from the eye.

22. Gordon (1904) obtained from a case of conjunctivitis a non-virulent organism identical with the diphtheria bacillus in morphology and staining properties. (Plate XIV, fig. 2.) On *serum* it formed lemon yellow colonies and on *agar* a dry growth and yellow pigmentation. On *gelatin* the growth resembled that of the diphtheria bacillus, but was yellow. *Glucose broth* became acid. In broth no turbidity was caused and growth occurred as conglomerate crumbs.

Griffith (1901) from his observations on the bacteriology of the conjunctiva considered that it would be possible "to separate from the eye a complete series of bacilli beginning with the short regular form not producing acid in glucose and non-virulent and ending with the typical diphtheria bacillus pathogenic to guinea-pigs."

He describes in detail three diphtheria-like organisms, not including the Xerosis bacillus, all of which are non-pathogenic.

23, 24. Two of these three resemble the Xerosis bacillus closely in all respects, the first (No. II.) differing mainly in producing a light brown growth on serum and the second (No. IV.) (probably a non-virulent diphtheria bacillus) in producing yellowish colonies on agar, and acid in broth lactose and glucose broth.

The third diphtheroid organism (No. V.) differs considerably.

25. Morphologically it is a short oval bacillus with a central unstained interval, showing polar bodies, retaining Gram's stain and producing "irregular soft opaque white colonies on serum, and white opaque spherical colonies on *agar*. *Gelatin* stroke cultures show a raised, opaque, porcelain, white, coarsely granular growth with lateral expansions. *Broth* becomes slightly tinted and has a white deposit. *Lactose* broth becomes slightly and *glucose* broth strongly acid. A little *indol* is produced but no *gas*. *Milk* becomes acid but is not coagulated. On *potato* a dry yellowish white growth occurs. Non-pathogenic.

Diphtheria of the ear.

Various lesions of the ear due to the presence of the diphtheria bacillus have been reported, and several of these reports are accompanied by satisfactory bacteriological evidence. Although the occasional presence of the true diphtheria bacillus in the ear discharges of patients suffering from diphtheria and others has been fully established, the frequency of its occurrence in these conditions is unknown. Many of the bacteriological reports are unsatisfactory, depending either on a few cultural characters, or on morphology alone, and several of them probably refer to other organisms.

---

[1] Peters (1897), Eyre (1897), Stephenson (1898), Heimersdorff (1898), Franke (1898), Axenfeld (1898), Lawson (1899), Griffith (1901), Gordon (1903). Schanz (1899) regards it as a non-virulent diphtheria bacillus.

Newsholme (1904) cultivated diphtheria bacilli from the ear discharges of a contact and two convalescents. In one instance the organism was tested and found to be virulent. Graham-Smith (1904) has observed altogether three instances of otorrhoea associated with fully virulent diphtheria bacilli. In one instance the bacilli persisted for 66 days and were virulent when tested on the 53rd day. Gordon (1903) once isolated virulent diphtheria bacilli from an ear discharge. In another case he discovered an organism which differed slightly in cultural characters and was non-virulent. Williams (1901) examined 62 ear discharges of scarlet-fever patients and discovered diphtheria-like bacilli in eight. Of these four were carefully tested, and all formed acid in sugar broth, but produced no effects on inoculated animals. Grixoni (1896) found non-virulent diphtheria-like organisms in a case of violent inflammation of the auditory meatus, and Schilling (1904) observed diphtheria-like bacilli in an ear discharge, which were apparently non-pathogenic to guinea-pigs.

Tobey (1906) obtained from the middle ear a diphtheria bacillus which produced acid with dextrose, but not with lactose or mannite. 2 c.c. of a broth culture injected subcutaneously caused an abscess in a guinea-pig.

Biernacki and Heanley (1906) investigated 24 cases of ear discharge in diphtheria patients. In 20 cases diphtheria bacilli were found. Of the four strains tested for virulence one was virulent and three non-virulent.

Table showing the virulence of diphtheria bacilli found in ear discharges.

Observers	Cases examined	Bacilli virulent	Bacilli non-virulent
Graham-Smith (1904)	3	3	0
Newsholme (1904)	1	1	0
Gordon (1903)	2	1	1
Biernacki and Heanley (1906)	4	1	3
Schilling (1904)	1	0	1
Williams (1901)	4	0	4
Grixoni (1896)	1	0	1
Tobey (1906)	1	0	1
	17	6	11

Diphtheria bacilli have also been reported without any remarks on their pathogenic properties by the following observers:

Scheller (1905) reports diphtheria bacilli in eight cases of post-diphtheritic otitis media. Funke (1901) studied the bacteriology of 76 cases of otitis media and in six he found diphtheria bacilli, in one the xerosis bacillus, and in six pseudo-diphtheria bacilli. He makes no mention of his methods, and his cases seem to have had no connection with diphtheria. He concludes from his observations that diphtheria bacilli are more commonly present in otorrhoea than is generally believed. Green (1901) examined bacteriologically 101 cases of acute suppuration of the tympanum and found diphtheria bacilli in four of them, twice in pure culture. This observer also investigated 144 cases of mastoiditis on which operations were performed, and found diphtheria bacilli in one instance. Councilman, Mallory and Pearce (1900) examined the middle ear in 144 fatal cases of diphtheria and observed diphtheria bacilli in 38 cases. They also cultivated diphtheria bacilli in one instance from an ear discharge. Wolff (1895) examined the middle ear in 22 fatal cases of diphtheria and found diphtheria bacilli in six. Councilman (1894) records the finding of diphtheria bacilli in three cases of otitis media, one following diphtheria, and Marsden (1901) in two cases following scarlet fever. Podack (1895) reported diphtheria bacilli in the ear discharges of two cases following measles, and Jacob (1905) found diphtheria bacilli both in the ear and nasal discharges of a child suffering from diphtheria. Leary (1897) and Kutscher (1895) each record one case.

Diphtheria-like bacilli from the ear.

Graham-Smith (1904) in the discharges from the ears of 10 scarlet-fever patients found on three occasions an organism resembling the diphtheria bacillus, described below as the *Bacillus auris*. From the ear discharges of three of these scarlet-fever cases and from the normal ears of 13 out of 20 persons working in the laboratory, another diphtheroid organism, *Bacillus ceruminis*, was isolated. Of all the ears examined, therefore, 66·6 % contained diphtheroid organisms.

26. *Bacillus auris.* (Plate XVI, fig. 3.)

Origin. From the ear discharges of three scarlet-fever patients.

On *serum* the colonies closely resemble those of the diphtheria bacillus, but grow more slowly. After 48 hours' growth the colonies are medium-sized. After 30 hours' growth the organisms are of various sizes, the majority of over medium length with darkly staining ends. They stain well, showing well marked segments, with intervening light bands. In the longer forms several segments occur. Nearly all, even the shorter forms, are well curved and have a tendency to be clubbed. A few pear-shaped forms were met with. They are non-motile, retain the stain by Gram's method, and show several well marked polar bodies by Neisser's stain. In subcultures the resemblance to diphtheria bacilli is still more marked. Nearly all the specimens are long, well segmented, and show several good polar bodies. The general arrangement in the field is similar to that of the diphtheria bacillus. On *agar* slopes in 24 hours the colonies are small, round, gray and dome-shaped. The organisms are short, curved and clubbed with well-marked polar bodies. Agar stab cultures in 24 hours show a smooth, moist, white surface growth, and a good growth of discrete round colonies along the line of puncture. On *gelatin* stabs in three days there is an irregular, granular, white surface growth faintly marked by concentric rings, and good growth in the depth. On *potato* in 24 hours there is a slight brownish-yellow growth, which in 48 hours becomes extensive, soft, yellow and glistening. The organisms are short and oval, and stain well with large terminal polar bodies. *Broth* in 48 hours is slightly cloudy with a white stringy deposit. In *glucose broth* a copious, finely granular deposit occurs, and the reaction is very markedly acid. *Milk* remains unchanged. *Indol* is formed. The organism is non-pathogenic.

These organisms closely resemble the diphtheria bacillus in morphology and staining characteristics, but differ, more especially, in their growth on gelatin, potato, and broth.

27. *Bacillus ceruminis.* (Plate XVI, fig. 4.)

Origin. Thirteen specimens were obtained from normal ears, and three from the ears of scarlet-fever patients. Eleven of these were fully investigated. The organisms appeared to be more plentiful when ceruminous secretion was present on the swabs. From this circumstance the name has been given.

On *serum* in 24 hours the growth is scarcely visible, but in 48 hours small, round colonies, indistinguishable from those of the diphtheria bacillus, are formed. After 72 hours the colonies are medium sized to large. Two forms slightly different in morphology were noticed. (*a*) In 30 hours long, thin and curved, and uniformly stained, with small terminal polar bodies. In 72 hours the bacilli are longer, more curved, well segmented, and with well marked polar bodies. (*b*) Medium length, slightly curved, uniformly stained, but markedly clubbed, with large polar bodies in a few specimens. After 72 hours the appearance of the two forms is similar. These organisms are non-motile, and retain the stain by Gram's method.

On *agar* after 24 hours the colonies are small, round, gray, and dome-shaped, but later become large and white. The organisms are of medium length, curved, often clubbed, fairly well segmented, and show good polar bodies. On agar stabs a small, white, moist surface growth is present in 24 hours, which after 48 hours' growth is often lightly marked with concentric rings. A confluent growth takes place in the line of puncture.

No growth was obtained on *gelatin*. On *potato* in 24 hours a whitish to yellowish-white growth occurs, later becoming abundant and yellow. After about 10 days the growth has a dry granular appearance. The organisms are mostly short, slightly curved, and clubbed. *Broth* remains clear and a small, stringy, white deposit is formed. Cultures of this organism in the serum medium of Hiss containing glucose, galactose, lactose, dextrin, laevulose, maltose, glycerine, mannite and saccharose form no acid even after 10 days' cultivation. In *glucose broth* the deposit is granular and the reaction neutral or alkaline. *Milk* remains unchanged and no *indol* is formed. They are non-pathogenic.

In morphology and staining characters these organisms closely resemble the diphtheria bacilli shown in Plate VI, fig. 6, but differ in the rate of their growth on serum, their characters on agar, potato and broth, and in producing no acid in glucose broth and no growth on gelatin.

"The results of these observations show that a species of non-pathogenic organism, almost indistinguishable from the diphtheria bacillus in morphology, is present in the majority of normal ears, and that another species also resembling the diphtheria bacillus is frequently present in the ear discharges of scarlet-fever patients. Consequently it seems highly probable that many of the organisms found in ear discharges and considered to be diphtheria bacilli, belonged to other species."

28. Hamilton (1904, No. 13) isolated from the ear discharge of a scarlet-fever patient an organism, which showed Neisser's granules, and in morphology closely resembled the typical diphtheria bacillus. It produced an abundant creamy growth on *serum*, and liquefied the medium. On *glycerine agar* there was an abundant colourless growth, and on *potato* an abundant yellow growth. *Broth* remains clear and becomes acid—non-pathogenic.

29. Hamilton (1904) also obtained a diphtheria-like bacillus in pure culture from the ear of a scarlet-fever convalescent who developed otitis media. After 18 hours' growth on *serum* short and long deeply staining forms as well as some barred and a few granular forms occur. The granules are well exhibited by Neisser's method. Serum is rapidly liquefied, and a purple growth is produced both on *agar* and on *potato*. *Broth* becomes acid and is not rendered cloudy. Injections of pure cultures rapidly kill guinea-pigs. They are protected by Ruediger's serum, but not by antitoxin.

30. De Simoni (1899) isolated from the discharge in a case of chronic pustular otitis slightly curved bacilli with rounded ends, which showed well defined polar granules but did not retain Gram's stain. In young cultures a few wedge-shaped forms were seen, but in old cultures they became more numerous. On *serum* small, raised, moist, white colonies, and on *agar* large, white, smooth colonies are produced. Good growth occurs on *potato*. *Milk* becomes coagulated in six days, and *broth* becomes clouded with a flaky deposit. They produce acid from glucose and are non-pathogenic.

31. Davis (1899) found in 10 out of 12 cases of otitis media following scarlet fever polymorphic diphtheria-like bacilli, some specimens being long and irregularly stained, others short and evenly stained. The organisms were non-motile and produced whitish colonies with uneven edges and dark centres on *agar*, and rendered *glucose broth* acid, and on *serum* grew like the diphtheria bacillus. Subcutaneous injections of 2 c.c. of broth culture of 9 strains into guinea-pigs produced no effect. One strain was pathogenic (septicaemia) and the animals were not protected by antitoxin.

32. Harrison (1907) isolated a diphtheria-like bacillus from an old case of chronic suppuration of the middle ear. The organism stained by Gram's method, showed polar bodies, and produced acid in *broth*. It differed from the diphtheria bacillus in producing a thick and creamy growth on *serum*, and a thick opaque yellow growth on *agar*. The agar was gradually coloured brown and eventually dark chocolate.

Marzinowsky (1900) obtained a non-virulent diphtheria-like organism from ear wax which retained Gram's stain, and showed polar bodies by Neisser's method.

Warnecke (1900) in two cases of ear disease, and Schilling (1904) in a case of acute otitis media, found non-virulent bacilli closely resembling diphtheria bacilli in other respects.

Forbes (1903) found diphtheria-like bacilli in 32 out of 40 ear discharges, derived from scarlet-fever patients. He was at first inclined to think that these were genuine diphtheria bacilli, but after further investigation abandoned this opinion (1904). Eleven of these bacilli were carefully examined, and all formed acid in *glucose broth*, and the majority produced visible growths on *potato*. Two were pathogenic to guinea-pigs, but at the autopsies none of the usual signs of death from experimental diphtheria were observed. He concludes by stating that "the diphtheroid bacilli differed from each other in many ways and evidently belonged to several groups. These bacilli cannot at present be classified as true diphtheria bacilli, neither do they belong to the commonly recognised varieties of pseudo-diphtheria bacilli."

Gordon (1901) studied seven cases of scarlatinal otorrhoea and found diphtheria-like bacilli in five. In one of these cases a virulent diphtheria bacillus was also found. All the diphtheria-like bacilli were non-virulent to guinea-pigs and all retained Gram's stain. Two strains caused litmus *milk* to become acid, while the other three made it alkaline.

Hamilton (1907) has recently made extensive investigations on the diphtheria-like bacilli found in suppurative processes, especially otitis media, studying 52 examples of miscellaneous suppuration and 142 of otitis media. The results are given in the following table:—

Disease	No. of cases examined	Diphtheria-like bacilli found in	Percentage infected	
Acute scarlatinal otitis media	43	31	72	36 °/o
Chronic ,, ,, ,,	9	5	55	
Acute non-scarlatinal otitis media	19	4	21	
Chronic ,, ,, ,,	71	11	15	
Miscellaneous suppurations	52	11	21	

"All the cases mentioned in this list as yielding diphtheria-like bacilli contained these bacilli as the predominating organism. Those in which only a few were found were ruled out as doubtful. In nine of the acute scarlatinal cases this bacillus was found in pure culture, in 16 more there were only a few colonies of other organisms."

She states that the 51 strains isolated from cases of otitis media and six from other lesions fall into two clearly defined groups.

Group 1 included 40 strains, which apparently resembled Hofmann's bacillus in morphology and in their growth on agar and potato. They produced acid from dextrose and saccharose, but not from maltose, lactose, or dextrin. Twelve strains were inoculated into guinea-pigs and of these nine proved to be non-virulent. The other three produced septicaemia and death in 24–48 hours. The animals were not protected by antitoxin.

Group 2 included 11 strains isolated from cases of otitis media. These organisms were long, segmented and clubbed and retained Gram's stain. All produced acid from dextrose and maltose, most (60 %) from dextrin, and a few (10 %) from lactose. Saccharose was not fermented. Four out of seven strains killed guinea-pigs. In one case the control animal was protected by antitoxin.

Hamilton says that "it is evident that there is no hard and fast line between this group and the true Klebs-Loeffler bacillus and when the organisms are non-virulent it is impossible to separate the two."

The *serum* of a rabbit immunised against one type is bacteriolytic for that type, but not for the other. Neither group was acted on by the *serum* of a rabbit immunised against the "Ruediger bacillus."

The discovery of diphtheria-like bacilli in ear discharges has led several recent writers to the conclusion that true diphtheria bacilli are often the cause of these lesions, and that the disease is frequently spread by persons suffering from them. The observations which have just been quoted show, however, that this conclusion is not at present justified, since they prove that diphtheria-like bacilli of various kinds, many of them very closely resembling true diphtheria bacilli and some pathogenic for animals, are very common in these conditions, but that true virulent diphtheria bacilli are rare.

In investigating these conditions it is necessary therefore to prove the identity of the organisms both by cultural tests and by inoculation experiments, including observations on control animals treated with antitoxin, before making a positive diagnosis of the presence of diphtheria bacilli.

The results of those workers who have investigated a series of cases are given in the following table (p. 373).

From this table it can be seen that diphtheria-like bacilli are present in at least 30 % of ear discharges. Of the 61 strains tested on animals 45 (73 %) proved to be non-virulent, six (10 %) were true virulent diphtheria bacilli, and 11 (18 %), though pathogenic, were not true diphtheria bacilli.

Further observations on this subject are required.

Cutaneous Lesions.

The majority of the recorded cutaneous lesions associated with the presence of diphtheria bacilli have occurred in persons suffering from diphtheria. A few instances, however, have been noted of cutaneous lesions due to the presence of diphtheria bacilli, whose identity has been fully proved, in persons who did not harbour diphtheria bacilli in their throats or noses. So many lesions of the skin, associated with the presence of diphtheria-like bacilli, have been described that it seems scarcely necessary to insist that every means of identification must be employed before organisms, found in such situations, can be considered to be true diphtheria bacilli. In many of the recorded cases, however, very insufficient proofs of the identity of the bacilli have been given.

Table showing the prevalence of diphtheria-like bacilli in ear discharges.

Observer	Cases examined	Diphtheria-like bacilli	Organisms tested on animals	True virulent diphtheria bacilli	Pathogenic—Antitoxin no effect	Non-pathogenic
Davis (1899)	12	10	10	0	1	9
Williams (1901)	62	8	4	0	0	4
Gordon (1901)	7	5	5	1[1]	0	5
Forbes (1903)	40	32	11	0	2[2]	9
Graham-Smith (1904)	13	6	6	3	0	3
Hamilton (1904)	2	2	2	0	2	0
Biernacki and Heanley (1906)	24	20	4	1	0	3
Hamilton (1907)	142	51	19	1	6	12
Wolff (1895)	22	6				
Councilman, Mallory and Pearce (1900)	144	38				
Funke (1901)	76	13				
Green (1901)	101	4				
	645	195 (30·2 %)	61	6	11	45

Cutaneous Lesions of the Face and Neck.

Fully virulent diphtheria bacilli have been found by Steffens (1900) in a case of gangrene of the eyelid, by Hála (1900) in an abscess of the eyelid, by Jéz (1895) in the fluid from the vesicles of *herpes labialis* in a patient suffering from diphtheria, and by Dávelos (1894) in the fluid of impetiginous lesions covering the face and neck of a baby.

In a number of other cases diphtheria bacilli have been described, without any record of their pathogenic properties.

---

[1] Diphtheria bacilli and diphtheria-like bacilli were present in one culture.

[2] The animals showed none of the characteristic lesions of experimental diphtheria.

Councilman, Mallory, and Pearce (1900) found diphtheria bacilli by culture in four out of 10 cervical abscesses developing in the course of diphtheria, and in one case of a membranous condition of the skin near the ear following an ear discharge. Prescott (1898) records a remarkable case. A child recovering from diphtheria developed a swelling in one of the glands in the neck, and a plaster was applied which produced a small blister. A false membrane later formed on the dead epidermis, and cultures showed diphtheria bacilli though none were present at the time in the nose or throat. Todd (1898) records the presence of diphtheria bacilli in a pustule of the face complicating diphtheria, Hayward (1895) in a lesion of the upper eyelid, Kanthack and White (1895) in membranous phagadaenic ulcers of the neck, and Kanthack and Stephens (1896) in several cutaneous membranous sores of the face and neck developing during the course of diphtheria.

Cutaneous Lesions of the Body.

Schottmüller (1895) isolated non-virulent diphtheria bacilli from the pus of an inguinal abscess and from the throat of a boy suffering from diphtheria, and Waelsh (1899) describes diphtheria-like bacilli, pathogenic for rabbits and guinea-pigs, in a serous and pustular eruption of the skin of the trunk. Pitts (1897) records an instance of diphtheria of the umbilicus in a child 14 days old, and Caddy (1893) an instance of diphtheria of the nipple and areola of a woman suckling an infant, which shortly afterwards developed the disease. In the first case no animal inoculations were made, and in the second no bacteriological examination was undertaken. Wright (1894) found diphtheria bacilli in one case of *fistula in ano*, and Müller (1891) in a pustule on the nates.

Cutaneous Lesions of the Hands.

Williams (1902) isolated virulent diphtheria bacilli from a membrane on the finger and from the throat of the same patient, and Abel (1894) from a lesion on the finger of a girl suffering from diphtheria. Müller (1899) and Seitz (1900) have also isolated virulent diphtheria bacilli from lesions of the fingers.

Heelis and Jacob (1906) found diphtheria bacilli in an ulcer on the dorsum of the hand. The patient later infected the outer canthus, and from this the disease spread to the conjunctiva.

A number of cases are also recorded without mention of animal experiments. Tavel (1902), Councilman, Mallory, and Pearce (1900), and Leary (1897) record one instance each, Park, Todd (1898) two cases, Garratt (1904), and Brunner and Hau (1900) three cases, and Wright (1894) seven cases. Two out of Garratt's three cases were suffering from scarlet fever, but diphtheria bacilli could not be demonstrated in the nose or fauces.

Cutaneous Lesions of the Lower Extremity.

Bernard and Jacob (1903) found virulent diphtheria bacilli in gray ulcerating patches on the thigh of a soldier. None were discovered in cultures from the nose and throat. Heelis and Jacob (1906) discovered virulent diphtheria bacilli in two cases of ulceration of the foot, thought to be due to chilblains. Similar organisms, which were not tested on animals, were found in an ulcer of the heel. All these cases occurred at the same time amongst the inmates of an orphanage. Bolton and Brewer (1905) cultivated diphtheria bacilli from a sore, showing a black central scab surrounded by sloughing portions of dirty white skin, on the groin of a girl. No membrane was ever formed. Animal inoculations are not recorded. Todd (1898) briefly records the finding of diphtheria bacilli in two pustules of the foot and two of the toe, complicating cases of diphtheria.

Without mentioning the part affected the discovery of diphtheria bacilli in cutaneous lesions is recorded by Tavel (1903) (abscess), Schick and Ersettig (1903), Gordon Sharp (1898) (two cases), and by Glucksmann (1897) (five cases of eczema). Leary (1896) found diphtheria bacilli in an abscess which developed about a hair follicle two days after an autopsy on a case of diphtheria. The abscess remained local, and a pure culture of the diphtheria bacillus was obtained from it which killed a guinea-pig in 48 hours.

Wound Diphtheria.

Several of the instances of cutaneous diphtheria, which have been mentioned, were due to the infection of insignificant wounds (Park, Pitts, Abel, etc.). Besides these a number of examples of infection of more extensive wounds have been recorded: Tavel (1902) mentions a case of infection of the operation wound for spina ventosa, Brunner (1893) three cases of wound infection, in one of which virulent, and in the other two non-virulent, diphtheria bacilli were found, Spronck (1892) three examples of infection of tracheotomy wounds. Treitch and Abel (quoted by Flexner, 1895) and Scheller (1905) also mention cases of wound infection.

It must be pointed out, however, that cases of wound infection are not common, and that many of the membranous conditions following the infection of wounds have been proved by numerous observers to be due to other bacteria.

Diphtheria-like bacilli from the skin.

Diphtheria-like bacilli have been frequently described as occurring in various lesions of the skin.

A. *Vaccinia and variola.*

33. Brown (1903) isolated diphtheria-like bacilli from three cases of vaccinia with oedema, but could not find them in uncomplicated cases. The organisms are curved, of irregular width, stain faintly and have rounded ends, and contain darkly stained rounded dots, at intervals. The arrangement of the bacilli is similar to that of the diphtheria bacillus. They retain Gram's stain and show a few polar bodies by Neisser's method. On *agar* grayish-white colonies are produced which coalesce after further growth. In *glucose broth* there is a white deposit and the medium remains neutral. On *potato* a yellowish growth occurs. No growth occurs on *gelatin*, and *milk* is not clotted. One of the inoculated guinea-pigs suffered from a small patch of oedema followed by an ulcer, but another showed no reaction.

34. Nakanishi (1900) found in the pustules of vaccinia in children and calves an organism resembling Hofmann's bacillus in morphology with a central light band and darkly staining ends, which he calls the *Bacillus variabilis lymphae vaccinalis*. On *serum* the colonies are pale yellow, and circular, and grow to a considerable size. On this medium some of the bacilli are short and evenly stained, some are spindle shaped and some segmented, and some have polar bodies. Long segmented bacilli are frequent and branching forms occur occasionally on *agar* and *potato*. Growth on *gelatin* is poor. *Broth* is at first clouded, but later becomes clear with a granular deposit and slight surface film. Good growth occurs in *milk*, and the medium is not coagulated. The growth on *potato* is poor. The organism is pathogenic to rabbits and guinea-pigs, in large doses intraperitoneally, but not to mice.

35. De Simoni (1899) obtained from smallpox pustules bacilli which lie in irregular masses and show small granules in their protoplasm. They are seldom curved and usually have one thick rounded end and one narrower and more pointed end. They retain Gram's stain. Stroke cultures on *serum* after 24 hours show a uniform, moist, lustrous, raised growth, which later becomes thicker. On *agar* the colonies tend to coalesce and produce a thick moist growth. On *gelatin* well marked growth occurs. *Broth* becomes clouded and has a thick flaky deposit.

On *potato* there is a thick, moist, raised, luxuriant growth. *Milk* is not clotted. In *glucose broth* acid is produced. Non-pathogenic.

Besser (1893) and Sanfelice and Malato (1903) have also described diphtheroid organisms in variola, and the latter authors, Neisser (1888) and Laudmann (quoted by Galli-Valerio, 1904) in vaccinia.

36. Galli-Valerio (1904) isolated from *vaccine lymph* a diphtheroid organism, *Corynebacterium vaccinae*, 2—4 μ in length, which shows polar bodies by Neisser's stain, but does not retain Gram's stain. The majority stain evenly by methylene blue, but some show lightly stained patches, and some segmentation. The organism is non-motile, and is apt to be arranged in clumps. On *serum* in 24 hours small white colonies are formed, which later become yellowish. On *agar* small, white, raised colonies surrounded by a clear zone are formed. Later the centre becomes prominent and the edges irregular, and the whole surface becomes covered with irregular bosses. On *gelatin* at 20° C. a similar growth is formed on the surface, but there is very little growth in the depth. No liquefaction occurs. On *potato* a slight grayish white growth occurs. *Broth* at first becomes slightly cloudy, but later becomes clear. A whitish surface growth and flocculent deposit are formed. In *milk* the organism grows well and the medium does not become coagulated. *Indol* is formed, but neither *glucose* nor *lactose* is fermented. Mice and rabbits are not affected by subcutaneous injections. One of the inoculated guinea-pigs developed a small abscess in which the organism was found in pure culture.

37. Klein (1897) isolated from glycerine emulsions of smallpox crust an organism, which he called *Bacillus xerosis variolae.* The organism is rather shorter than the diphtheria bacillus but resembles it closely in morphology. On *agar* very minute colonies are formed which have a raised centre, irregular margin and granular surface. Very little growth occurs on *gelatin.* It is non-pathogenic to rodents and calves.

38. Klein (1897) also isolated from the same material another diphtheroid organism which he called *Bacillus albus variolae.* The bacilli from early agar cultures are very small, but those from older cultures resemble diphtheria bacilli very closely. Amongst these forms a few are to be seen with a conspicuous and deeply stained sheath. On *agar* the colonies are at first small and translucent, but after two days' growth become pure white, large, smooth, raised, and moist looking. The colonies are coherent and satisfactory microscopic preparations are not easy to obtain. Very little growth occurs on *gelatin.* It is non-pathogenic for rabbits and guinea-pigs, but cutaneous inoculations produce vesicular lesions in calves.

39. Levy and Fickler (1900) obtained from calf lymph two varieties of a diphtheroid organism, which they term *Corynebacterium lymphae vaccinalis.* One variety produces a yellowish, and the other a whitish growth on Loeffler's serum. On Loeffler's *serum* a thick, rough, dry growth is formed, and the organisms in young cultures include small wedge-shaped and longer diphtheria-like forms. On *agar* small, gray, irregular colonies with yellowish centres are produced, and a good growth occurs on *gelatin.* *Broth* becomes cloudy and there is a granular deposit. There is no growth in *milk* or on *potato.* Injections cause small abscesses in guinea-pigs and kill mice in 6—7 days with the formation of abscesses.

All the above investigators lay stress on the statement that the organisms they describe belong to the diphtheria group.

B. *Diphtheria-like bacilli in leprosy.*

40. Babes (1899) and a number of other observers have isolated diphtheria-like bacilli from cases of leprosy. Babes obtained them from 12 cases, and Spronck (1898), Levy (1897 and 1899), Czaplewski (1898), Baranikow (1899), Teich (1899), and Kedrowski (1901) have all obtained cultures resembling those of Babes. The discovery by Dean (1905) of a diphtheroid bacillus in rats suffering from a leprosy-like disease is interesting in connection with these observations. (See p. 291.)

The organisms closely resemble diphtheria bacilli in appearance, the majority being long and slightly curved, especially at their extremities. The longer forms also show transverse banding. They stain well with methylene blue, and exhibit metachromatic granules. Growth can be obtained on *serum*, *glycerine agar*, and *agar*, the colonies resembling those of the diphtheria bacillus. They are non-pathogenic to the ordinary laboratory animals, mice, guinea-pigs, rabbits and fowls.

C. *Other skin lesions.*

Peters (1897) observed non-virulent diphtheria-like bacilli in three cases of impetiginous eczema, Paffenholz (1895) in impetigo, Warnecke (1900) in a progressive phlegmonous condition following an ear discharge, and Neisser (1888) in an ulcer of the leg. These authors regarded the organisms they found as Xerosis bacilli.

De Simoni (1899) has also described diphtheria-like bacilli, apparently belonging to different species, in pustules, seborrhoeic eczema, dry eczema, and ringworm. These all formed acid from *glucose*, grew well on potato, and most of them caused clotting in milk.

Bergey (1898) isolated non-pathogenic acid-forming diphtheria-like bacilli, having the same cultural characters as those he cultivated from urine (see p. 386), from the patches of a scaly skin eruption and from an abrasion of the knee. Cobbett (iv. 1901, p. 244) also mentions that he has occasionally met with diphtheroid bacilli from the skin.

Diphtheria of the Female Genital Organs.

Diphtheritic lesions of the female genital organs involving the labia, or vagina, or both, have frequently been recorded, but in most of these cases the diagnoses have been based on clinical signs alone. Many of these records show that diphtheria of the mouth or nose was present at the same time. Even in those instances in which bacteriological examinations have been made, the proofs are in many cases unsatisfactory owing to the lack of animal experiments. The following table summarises the principal records of cases in which bacteriological investigations have been conducted.

Observer	Cases reported showing diphtheria bacilli	Presence in throat or nose	Virulence
Schwab (1904)	2	Present	1 virulent 1 non-virulent
Williams (1902)	1	Present	Virulent
Müller (1899)	1	Absent	Virulent
Freymouth & Petruschky (1898)	1	—	Attenuated
Longyear (1897)	6	Present in one	—
Salmon (1904)	1	Present	—
Erikson (1905)	1	Present	—
Leick (1900)	1	Absent	—
Coues (1897)	1	Present	—
Hewlett & Nolan (1896)	1	Present	—
Bumm (1895)	1	Present	—
Glucksmann (1897)	3	—	—
Favre (1890)	2	—	—
Ware (1900)	1	—	—
Scheller (1905)	1	—	—
Elsner (1898)	1	—	—
Pearce (1898)	1	—	—
Williams (1898)	1	—	—
Cioffi (1897)	1	—	—
Haultain (1897)	1	—	—
Stahl (1895)	1	—	—
Nisot (1896)	1	—	—

Salmon's (1904) case is of especial interest in that patches of membrane were first noted on the labia minora and near the meatus, without local or constitutional disturbance, in a woman admitted into a ward in which an outbreak of diphtheria had occurred. Seven days later during a routine examination of healthy persons diphtheria bacilli were found in her throat. The following day patches of membrane were found on the tongue, lips, palate and mucous membrane of the cheek. The day after a dense white membrane was present on the rectum, vulva and vagina, which a few hours later extended to the cervix. At the same time an abrasion on the cheek also developed a membrane. The patient died 15 days after the lesions were first noticed. Diphtheria bacilli were cultivated from all these lesions.

Of the cases recorded above, six occurred in adults, sixteen in puerperal women, and eight in girls.

Diphtheria-like bacilli from the female genital organs.

41. Foulerton and Bonney (1903) in two cases of puerperal fever isolated diphtheria-like bacilli from the uterus. These organisms are rather longer and coarser than diphtheria bacilli. Some are segmented, others uniformly stained.

They retain Gram's stain but do not show granules with Neisser's stain. They produce a scanty growth on *agar* at 37° C. and after 24 hours' growth show a variety of forms—long beaded, spindle-shaped, pear-shaped, and coccus-like may be seen. Stroke cultures have a surface like ground glass. The growth on *serum* is like that on agar but more opaque. In *broth* it appears as a short evenly staining rod. No acid is produced in *glucose broth*, *milk* remains unchanged, and on *potato* there is a moist spreading layer. This organism differs from the diphtheria bacillus in its coarser shape, and the fact that it produces no acid in glucose peptone broth, and its lack of virulence for guinea-pigs and rabbits. The authors also observed these organisms in cases of catarrh of the cervix uteri.

42. Bergey (1898) isolated from a vaginal discharge a non-pathogenic, acid-forming bacillus corresponding in all respects to the bacillus he isolated from urine.

43. From another vaginal discharge Bergey isolated another segmented diphtheroid organism which produced a thick, moist, yellow growth on *serum* and *agar*, a visible growth on *potato* and a thick yellowish-white membrane on the surface of *broth*. *Glucose broth* became markedly acid after 72 hours' incubation. *Guinea-pigs* inoculated intraperitoneally with large quantities died, but did not show lesions characteristic of experimental diphtheria.

Hallé (1899) found diphtheria-like organisms, varying slightly in morphology, in different parts of the female genital canal, which reminded him of short diphtheria bacilli. All were totally non-pathogenic for guinea-pigs, rabbits and mice. Neisser (1888) isolated diphtheria-like organisms from the vagina, which differed from true diphtheria bacilli in being motile and growing well on potato. Robertson and McRae (v. 1905) isolated bacilli, morphologically identical with diphtheria bacilli, from the leucorrhoeal discharges of a number of female general paralytics.

Many other observers have also briefly mentioned the occurrence of diphtheria-like bacilli in these situations.

Diphtheria of the Male Genital Organs.

Well authenticated cases of diphtheritic lesions of the male generative organs, in which bacteriological examinations have been made, are rare.

McCollom (1897) describes the case of a boy, aged 4 years, suffering from nasal diphtheria who developed a membrane on the prepuce. Virulent diphtheria bacilli were isolated. The same author records a second case in a boy, aged 1 year, suffering from diphtheria of the fauces, who had previously been circumcised. The glans became red and oedematous but no membrane formed. Diphtheria-like bacilli were isolated, which proved non-virulent for guinea-pigs.

Post (1897) quotes the case of an adult who had nursed two cases of diphtheria. He developed a blister on the inner surface of the prepuce which caused acute phimosis. An incision was made and on this a membrane formed, and the prepuce later sloughed. On admission to the hospital no diphtheria bacilli were obtained in cultures, but diphtheria-like organisms were found in sections of the prepuce. Baranikow (1901) records a case of urethritis in an adult, in the discharge from which virulent diphtheria bacilli were discovered. Munn (1893) quotes the case of a baby, who was circumcised and developed a membrane on the wound, in which diphtheria bacilli were found. Both the mother and brother later suffered from diphtheria. Brunner (1893) discovered diphtheria bacilli in a case of phlegmon of the scrotum.

Diphtheria-like bacilli found in the male urethra.

44. Pfeiffer (1903) examined the urethra in 15 normal men and in 10 suffering from gonorrhoea. He found diphtheroid organisms by cultural methods 11 times in the former group and six times in the latter.

These organisms show a peculiar uneven staining by Loeffler's methylene blue, do not retain Gram's stain, and are neither alcohol nor acid fast. They grow well on *serum*, and produce punctiform, round, grayish white, smooth or slightly granular colonies on *agar* after 48 hours' growth. Growth also occurs on *gelatin*. *Broth* remains clear, and a granular sediment is produced and sometimes a slight surface film. There is a slight acid formation in *neutral broth*. *Milk* becomes acid in 2—7 days at room temperature, and a slight growth occurs on *potato*. *Animal* experiments were negative. Diphtheria agglutinating serum produced no results with six strains in various dilutions between 1 : 20 and 1 : 500.

45. The writer has also recently isolated diphtheroid organisms from the urethra in two cases simulating gonorrhoea, in which the gonococcus was not found, and from one case of gonorrhoea. In smears made from the discharge their morphology varies between short, uniformly staining and long, segmented bacilli. On *serum* colonies are formed after 24 hours' growth like those of the diphtheria bacillus, and the organisms are mostly long and curved and with many segments. Short forms are not uncommon. The majority are rather broader than diphtheria bacilli, and many have enlargements twice as thick as the rest of the bacillus, usually situated about half way between one end and the centre of the bacillus. Distinctly club-shaped forms are also common. They do not possess polar bodies. On *agar* and *glycerine agar* large, white, smooth, moist colonies are produced. A good growth of small, moist, white, round colonies occurs in 48 hours on *gelatin*, and *broth* becomes clouded, and a thin flaky film and large yellow deposit are produced. *Glucose broth* remains neutral. On *potato* an abundant, dry, granular, yellowish growth occurs in 48 hours. Subcutaneous injections of 3 c.c. of 48 hours' broth cultures produce no effect on guinea-pigs. (Plate XVI, fig. 6.)

Foulerton and Bonney (1903) observed in a case of phagadaena of the penis organisms similar to those described in two cases of puerperal fever (No. 41), but did not detect them in 56 cases of urethritis. Eastes (1903) records a similar organism, which was non-pathogenic and did not stain well by Neisser's method, in the discharge of a case of urethritis.

Robertson and McRae (v. 1905) found organisms, morphologically and culturally resembling diphtheria bacilli, but non-virulent, in material taken from the surface of the urethra in 22 consecutive cases of general paralysis in the male.

TABLE SHOWING THE PRINCIPAL CHARACTERS

Morphology	Gram	Neisser	Growth on serum	Growth on agar	Growth on gelatin	Growth on potato
A. Resembling diphtheria bacilli in all their main cultural characters.						
Segmented	+	+	Diphtheria-like	Gray colonies	—	—
,,	+	+	White colonies	,,	Little growth	Invisible growth
Segmented and non-segmented	+	+	Diphtheria-like	White colonies	,,	,,
Segmented	0		White colonies	White round punctiform colonies	,,	,,
,,		+	Diphtheria-like	Diphtheria-like	Diphtheria-like	,,
At first Hofmann-like, later segmented			,,	,,	,,	,,
Segmented	+	+	White	Dry pearly white colonies	Little growth	No growth
,,			Diphtheria-like	Diphtheria-like	,,	,,
,,	+	+	Diphtheria-like or dry	,,	,,	—
Hofmann-like		+	Yellowish colonies	—	,,	Little growth
B. Organisms differing from diphtheria bacilli mainly in the character of their growth on serum.						
Segmented	+	+?	Slow growth, scaly adherent colonies	Small gray adherent colonies	Little growth	Invisible growth
,,	+	+	Opaque white colonies becoming brownish	Small gray granular colonies	White coarsely granular growth	White dry growth
Uniformly staining	+	+	Coherent growth	White coherent	No growth	—
Beaded	+	0	Coherent sharply outlined colonies	—	,,	—
C. Organisms differing from diphtheria bacilli mainly (1) in the non-production of acid.						
Hofmann-like	+	0	Diphtheria-like	White colonies	Fair growth	Invisible growth
Segmented	+	+	Granular adherent colonies	Dry gray growth	—	—
,,	+	+	White filmy growth	White colonies becoming confluent	Colonies white or buff	No growth
Uniformly stained or segmented	0	+	White growth becoming yellow	White	Fair growth	Slight gray growth
Segmented	+	+	Diphtheria-like adherent	Gray	No growth	Invisible growth
,,	+	+	Diphtheria-like	,,	Little growth	,,
				(2) in the non-production of acid and the production of		
Segmented	+	0	Diphtheria-like	Moist white	White	Abundant dry yellow
Segmented or uniformly stained	+	0	Scanty growth	Scanty growth, surface like ground glass	—	Moist spreading layer
Segmented	+	+	Semi-transparent, later yellowish colonies	—	White, later citron coloured	Yellowish powdery

OF DIPHTHERIA-LIKE BACILLI.

Growth on broth	Acid	Gas	Milk		Indol	Virulence	Source	Name	Observer	See
			Acid	Coagulation						
Medium clear granular deposit	+					General septicaemia in guinea-pigs. Not protected by antitoxin	Human mouth	—	Davis (1899)	Page No. 354, **14**
Medium clear deposit of large granules	+		0	0	0	Non-pathogenic	,,	*B. maculatus*	Graham-Smith (1904)	349, **1**
Medium clear stringy deposit	+		+	+		,,	Nasal catarrh	*B. coryzae segmentosus*	Cantley (1896)	361, **19**
Medium clear granular deposit	+		+	0		,,	Normal and diseased male urethra	—	Pfeiffer (1903)	381, **44**
,, ,,	+					Produces abscesses in rats and guinea-pigs	Hepatised lungs of rats	*B. muris*	Klein (1903)	291
Medium clear deposit of large granules	+					Slightly pathogenic for young rats	Leprosy-like disease of rats	—	Dean (1905)	291
Medium clear granular deposit	+					Non-pathogenic	Urine	—	Bergey (1898)	386, **46**
Medium clear flocculent deposit	+		+	0	+	,,	Milk	—	,,	328
—	+				+	,,	Mouths of healthy & diseased pigeons	—	Macfadyean & Hewlett (1900)	297
Medium clear granular deposit	?		0	0		Pathogenic in large doses to rabbits and guinea-pigs	Vaccinia	*B. variabilis lymphae vaccinalis*	Nakanishi (1900)	376, **34**
Medium clear granular deposit	+?		0	0		Non-pathogenic	Normal eye	*B. xerosis*	—	363, **21**
,, ,,	+	0	+	0	+	,,	,,	—	Griffith No. IV (1901)	366, **24**
Medium clear coherent masses at bottom	+		0	0		,,	Human mouth	—	Gordon No. 7 (1903)	350, **2**
,, ,,	+		+	+		,,	,,	—	Gordon No. 8 (1903)	350, **3**
At first cloudy, later clear fine deposit	0	0	0	0	+?	Non-pathogenic	See p. 199	*B. Hofmanni*	—	—
Medium clear granular deposit	0	0	0	0	0	,,	Human eye	—	Griffith No. II (1901)	366, **23**
,, ,,	0	0	0	0	0	,,	Milk	"*Diphtheroid III*"	Eyre	329
Medium clear flocculent deposit	0		0	0	+	,,	Vaccinia	*Corynebacterium vaccinae*	Galli-Valerio (1904)	377, **36**
Medium clear granular deposit	0					,,	Normal eyes of dogs & guinea-pigs	*B. xerosis canis*	Graham-Smith (1904)	292
,, ,,	0				+	,,	Mouth of hen	*B. diphtheroides gallinarum*	,, ,,	297

a copious growth on potato.

Cloud and large yellow deposit	0					Non-pathogenic	Male urethra	—	Graham-Smith	381, **45**
—	0		0	0		,,	Female genitals	—	Foulerton & Bonney (1903)	379, **41**
Medium clear granular deposit	0		0	0	0	,,	Mouth and milk	"*Diphtheroid I*"	Eyre (1900)	329

TABLE SHOWING THE PRINCIPAL CHARACTERS

Morphology	Gram	Neisser	Growth on serum	Growth on agar	Growth on gelatin	Growth on potato
C (*cont.*). **Organisms differing from diphtheria bacilli mainly (2) in the non-production of acid and the**						
Uniformly stained	+	+	Slow growth, colonies diphtheria-like	Gray	No growth	Abundant yellow growth
Segmented	+	+	Raised centre with thin gray surrounding zone	White moist	,,	Abundant dry yellow growth
Beaded	+	+	—	White colonies coalesce	,,	Yellow growth
D. **Organisms differing from the diphtheria bacillus in producing abundant, generally coloured, growths**						
Uniformly staining	+	+	Diphtheria-like	Large white	No growth	Extensive moist yellow
Segmented	+	+	,,	White slimy	Large dry colonies	Extensive cream coloured
Uniformly staining	+	+	Moist yellow growth	Gray colonies	Moist growth	Dry yellow growth
,, ,,		0	Yellow growth	Yellow growth	—	Abundant yellow
Segmented	+	+	Diphtheria-like	Gray	No growth	,,
Uniformly staining	0	+	Moist white growth	Smooth white colonies becoming confluent	Good growth	,,
Hofmann-like	+	+	Soft cream coloured	White	Porcelain white growth	Dry yellowish-white growth
Granular	+	0	Abundant	Thick moist	Good growth	Abundant moist
Segmented			Moist yellow	Moist yellow	—	White
Segmented and uniformly staining forms		0	Yellowish growth	Yellow	Yellow	Yellow
E. **Organisms differing from diphtheria bacilli in liquefying serum or gelatin.**						
Slightly segmented, motile	+	+	Yellow colonies, medium slowly liquefied	Abundant moist yellow growth	Liquefied	Abundant white growth becoming yellow
Segmented	+	+	Gray colonies	Granular, adherent colonies	Slowly liquefied	—
,,	+	+	Abundant creamy, medium liquefied	Colourless growth	—	Abundant yellow growth
,,	+	+	Medium liquefied	Growth slow	No growth	No growth
F. **Organisms differing from diphtheria bacilli in liquefying gelatin and producing soluble pigments.**						
Uniformly stained		0	Medium liquefied	Grayish growth, medium becomes purple	—	Brown growth
Segmented		+	Medium rapidly liquefied	Purple growth	—	Purple growth
G. **Organisms differing from diphtheria bacilli in producing pigmented growths or soluble pigments.**						
"Sheath" form	+	+	Diphtheria-like or confluent	White	Medium made claret coloured	Pink colonies, medium pink
Segmented		0	Colourless growth	Abundant growth, medium becomes brown	—	Abundant gray
,,	+	+	Lemon yellow colonies	Dry yellow	Yellow	—
H. **Organisms differing from diphtheria bacilli in producing spores.**						
Segmented			White colonies which coalesce	Diphtheria-like	No growth	Moist growth

OF DIPHTHERIA-LIKE BACILLI (*continued*).

Growth on broth	Acid	Gas	Milk		Indol	Virulence	Source	Name	Observer	See
			Acid	Coagulation						
production of a copious growth on potato.										
Medium clear stringy deposit	0		0	0	0	Non-pathogenic	Normal ear	*B. ceruminis*	Graham-Smith (1904)	Page No. 369, 27
Thick surface film, flocculent deposit	0					,,	Mouth	—	Graham-Smith	352, 7
White deposit	0			0	0	,,	Vaccinia	—	Brown	376, 33
on potato.										
Medium clear stringy deposit	+					Non-pathogenic	Abscess of mouth	*B. diphtheroides citreus*	Graham-Smith (1904)	350, 5
Cloudy, granular deposit	+		+	+	+	,,	Mouth	*B. diphtheroides brevis*	,,	351, 6
Thick surface film, stringy deposit	+					,,	,,	—	Hamilton (No. 19)	
—	+					,,	,,	—	,,	352, 8
Cloudy, stringy deposit	+		0	0	+	,,	Ear	*B. auris*	Graham-Smith (1904)	368, 26
Cloudy, flocculent deposit	+			+		,,	,,	—	De Simoni (1898)	370, 30
Cloudy, granular deposit	+	0	+	0	+	,,	Normal eye	—	Griffith No. V (1901)	366, 25
Cloudy with flaky deposit	+			0		,,	Variola	—	De Simoni (1898)	376, 35
White membrane	+					Pathogenic for guinea-pigs in large doses	Vagina	—	Bergey (1898)	380, 43
Cloudy	+	+				Produces septicaemia; antitoxin does not protect	Mouth	—	Hamilton	357, 16
Cloudy, granular deposit	0	0		+	+	Non-pathogenic	Mouth	*B. diphtheroides liquefaciens*	Graham-Smith (1904)	353, 12
—	+			+	+	,,	,,	—	Gordon	350, 4
Medium clear	+					,,	Ear	—	Hamilton	370, 28
Little growth			+	+		Produces subcutaneous abscesses in guinea-pigs	Milk	*B. diphtheroides*	Klein (1901)	328
Cloudy	+					Non-pathogenic	Mouth	—	Hamilton	353, 13
Clear	+					Produces septicaemia; animals not protected by antitoxin	Ear	—	,,	370, 29
Cloudy	+	0			0	Non-pathogenic	Milk	*Diphtheroid II*	Eyre	329
,,	+					,,	Mouth	—	Hamilton	352, 9
Conglomerate crumbs	+					,,	Eye	—	Gordon	366, 22
Medium clear granular deposit	+					Non-pathogenic	Nose	—	De Simoni (1899)	362, 20

Diphtheria of the Anus and Rectum.

Diphtheritic lesions of the anus and rectum are very rare. Salmon (1904) mentions a case in which the patient was suffering from several other diphtheritic lesions. Wright (1894) found diphtheria bacilli in a case of *fistula in ano,* and Neisser (1893) in an inflammatory condition round the anus. Biggs (1893) also mentions an instance of diphtheria of the rectum. In none of the cases was the virulence of the organisms proved.

Diphtheria Bacilli in the Urine.

In a few instances the presence of diphtheria bacilli in the urine has been reported, but in no instance have the bacilli been satisfactorily identified. Barlow (1898) reports the case of a young woman suffering from diphtheria with membrane on both tonsils. On the third day dark coloured urine was passed, and bacilli resembling diphtheria bacilli in morphology and cultural characters were found. No virulence tests were made.

Bujwid (1897) found diphtheria-like bacilli in a sample of urine from a child suffering from tubercle of the kidney. In morphology and cultural characters the organisms resembled diphtheria bacilli, but the subcutaneous injection of 1 c.c. of a ten days' culture only caused strong local symptoms in a guinea-pig.

Diphtheria-like bacilli in urine.

Bergey (1898) isolated diphtheria-like bacilli from urine on several occasions (10). The urine was obtained both from healthy patients and from persons suffering from cystitis.

46. These organisms are short, slender rods showing a tendency to clubbing at the ends, which are stained more darkly. When stained with dilute methylene blue the bacilli show well marked segments. They retain Gram's stain, and show polar granules. On *serum* small round whitish colonies and on *agar* small round dry pearly-white colonies are formed. On *gelatin* there is very little growth, and on *potato* none. *Broth* does not become clouded but a slight granular deposit is formed. In *glucose broth* acid is produced. Entirely non-pathogenic to guinea-pigs.

Cerebro-spinal fluid.

Morrell and Wolf (1906) on two occasions obtained diphtheria bacilli during life from the cerebro-spinal fluid of a child suffering from general miliary tuberculosis and tubercular meningitis, but without any signs of diphtheria. The organisms were typical in morphology and cultural reactions, and produced acid from glucose and dextrin, but not from saccharose or inulin. Inoculated guinea-pigs died in 48–56 hours with gelatinous oedema and supra-renal reddening.

Head and Wilson (1899) isolated after death typical virulent diphtheria bacilli from the ventricles and cerebro-spinal fluid of a woman suffering from suspected rabies.

Non-virulent diphtheria-like bacilli have been isolated from the brain and spinal fluids by Johnson and Goodall (1902) and Robertson, McRae and Jeffery (1903).

Diphtheria bacilli have in some instances been separated from the organs of persons dead of the disease (see Chapter III, p. 99), and diphtheria-like bacilli have been found in the sputum and organs of persons suffering from general paralysis of the insane (p. 441).

Kruse and Pasquale (1894) isolated a non-pathogenic organism, having the cultural and morphological peculiarities of the diphtheria bacillus, from the pus of a liver abscess following dysentery. They gave it the name of *Bacillus clavatus.*

Summary.

A very large number of bacilli have been obtained from the secretions of healthy persons and from various lesions of the throat, nose, eye, ear, skin, and genitalia, as well as from various animals and from milk, which more or less closely resemble the diphtheria bacillus in morphology. Many of them also closely resemble it in staining reactions and in cultural characters. The majority are non-pathogenic to laboratory animals, but some are pathogenic. Of the latter a few produce septicaemia, and others less acute conditions. All these bacilli may be roughly divided into groups according to the differences they exhibit in culture. The members of one group, though derived from widely different sources, closely resemble true diphtheria bacilli in all respects except in their action on animals. Amongst this group may be placed the non-virulent diphtheria bacillus, the bacilli described by Robertson, Kruse and Pasquale, and many others, which apparently possess no cultural characters by which they can be separated from the diphtheria bacillus.

Other groups are peculiar in producing coherent or adherent colonies on serum, extensive, often coloured, growths on potato, liquefaction of serum or gelatin, soluble pigments, or in developing spores.

Another group of diphtheroid organisms produces no acid in glucose broth. According to the writer's experience true virulent diphtheria bacilli never produce gas or possess any of the above characters, and descriptions of virulent diphtheria bacilli possessing any of them are rarely to be met with. Bacilli which show any of the above characters may therefore be separated from true diphtheria bacilli.

Many of the organisms described possess no pathological significance, and appear to be harmless saprophytes. Their presence may, however, lead to erroneous diagnoses, and unjustifiable conclusions as to the nature of the disease, the method of treatment, and the distribution of diphtheria bacilli. A few produce severe lesions in man, which may be mistaken for diphtheria. Judging from the experiments of Ruediger, Hamilton and Horton such organisms are not very uncommon and their importance has not yet been sufficiently recognised.

In considering the distribution of diphtheria bacilli amongst non-contacts and the diphtheritic origin of various lesions, the frequent occurrence of diphtheroid bacilli in many parts of the body must be borne in mind, and all such organisms thoroughly tested before any final decision is given[1]. Outside the human body diphtheroid organisms have also been found in milk, on the normal and diseased mucous membranes of many animals, and in other situations. In dealing with organisms found under these conditions similar precautions are necessary.

[1] See also pp. 264, 265.

CHAPTER X.

DIPHTHERIA-LIKE DISEASES.

Characters of diphtheritic lesions. Organisms which have been found associated with non-diphtheritic membranous lesions of the mouth, nose and larynx: Streptococci, Staphylococci, Brison coccus, Pneumococcus, Bacillus of Friedländer, Bacillus coli, fusiform bacilli and Spirilla, Leptothrices, Yeasts, Syphilis.

BEFORE proceeding to describe the diseases which simulate diphtheria, it may be well to recapitulate briefly the various forms in which diphtheria may be met with in different parts of the body.

Diphtheria most commonly affects the posterior portions of the buccal cavity, producing in its typical form pseudo-membranes covering a part or the whole of the tonsils and the adjacent structures. Examples have, however, been encountered in which the membrane covered almost the whole of the surfaces within the buccal cavity, and on the other hand true diphtheria may show only very limited changes such as small membranous areas on the tonsils, white plugs in the crypts, or even simple redness on the tonsils without membrane. Diphtheritic membranes confined to the tongue have been noted by Trevelyan (1900), Thiercelin (1898), and Wharton (1895, bacilli virulent), and to the lips and gums by Trevelyan (1900) and Flexner and Pease (1895, two cases). Diphtheria bacilli have also been observed in noma by several observers (p. 348). Diphtheritic conditions of the larynx and trachea may also vary from extensive membranous formations to very slight lesions. Horne (1905), for example, mentions a case in which no diphtheria bacilli could be found during life, and were only obtained at the autopsy from the ventricle of the larynx.

In a similar manner diphtheria of the nose manifests itself in a variety of ways. Membranous or fibrinous rhinitis, acute or subacute catarrh, atrophic rhinitis in certain forms (Symes, 1903) and external rhinitis (Todd, 1898) may all be caused by virulent diphtheria bacilli.

Diphtheritic conjunctivitis has been reported by numerous observers, and virulent diphtheria bacilli have been encountered in ear discharges on several occasions. Membranous lesions of the external female genital organs, including the labia, vulva and vagina, and a few cases of lesions of the penis due to diphtheria bacilli have been recorded. Lesions of the skin in many parts of the body as well as abscesses and ulcers associated with the presence of virulent diphtheria bacilli have also been recorded. More rarely diphtheria bacilli have been observed to be present in lesions of the internal organs without external manifestations of the disease. Thus diphtheria bacilli have been cultivated from the brain in a supposed case of rabies by Head and Wilson (1899), from the cerebro-spinal fluid of a case of tuberculosis during life by Morrell and Wolf (1906), from the heart valves by Howard (1894), from a pneumonic lung by Ohlmacher (1895), etc.

The clinical manifestations of diphtheria are so divergent that the widest experience cannot hope to diagnose correctly on clinical grounds alone any but the more typical cases. Even in such cases mistakes are not infrequently made. The assistance of bacteriology is necessary before the identity of the less typical examples can be proved, and bacteriological methods should always be employed in the case of persons presenting throat or nose lesions, who have recently been in contact with diphtheria.

On the other hand a few examples do undoubtedly occur in which bacteriological evidence is at fault in the first examinations. In such cases the growth of the diphtheria bacilli in the culture tubes may be retarded or overwhelmed by the accompanying bacteria, or it may be inhibited by the presence of antiseptics, or from some other cause the bacilli may not develop. The first of these factors, however, seldom comes into action except in extremely foul ulcerative cases, and the proportion of erroneous bacteriological diagnoses from all causes is very small.

Even the anatomical condition of the membrane cannot be regarded as a certain proof of the nature of the disease. "As a rule the exudate of diphtheria is firmly incorporated with the underlying mucous membrane and cannot be removed without leaving a bleeding surface, at least until convalescence. The tissues surrounding the exudate are more or less inflamed and swollen. When other bacteria produce the irritant the exudate, except in the cases due to the bacillus described by Vincent, is usually loosely attached, collected in small masses, and easily removable. Exceptions, however, occur in both these diseases, so that in true diphtheria, the exudate may be easily removed, and in lesions due to other bacteria the exudate may be firmly attached" (Park, 1900).

Organisms which have been found associated with Non-diphtheritic Membranous Lesions of the Mouth, Nose and Larynx.

Streptococci. Numerous observers have recorded cases of membranous inflammation of the mouth in which streptococci have been the only organisms present. Prudden (1889) studied a series of 22 fatal cases, and Woodhead (1901) found streptococci to be the only organisms present in 565 out of 1960 cases diagnosed on clinical grounds as diphtheria, in which no evidence of diphtheritic infection was obtained.

Staphylococci. Examples of membranous inflammation due to staphylococci are of very common occurrence, and even limited outbreaks of this disease have been described.

Mixed infection with streptococci and staphylococci is a more common cause of pseudo-diphtheritic lesions than pure infection with either of these organisms. Woodhead, for example, found that 985 (50·2 %) of his 1960 cases of pseudo-diphtheria were caused by mixed infection with these two organisms.

Brisou coccus. This organism has been often observed in pseudo-diphtheritic conditions, and forms colonies resembling those of the diphtheria bacillus. Its characters have been described by Roux and Yersin. Chaillou and Martin (1894) mention cases due to this organism in which the membrane extended into the trachea necessitating tracheotomy. Stone (1904) has recently described a peculiar diplococcus occurring in acute inflammatory conditions of the throat accompanied by a severe toxaemia. In many cases a distinct white tenacious false membrane is present on the tonsils. The adjacent sides of the cocci are flattened and they show distinct metachromatic granules when stained by Loeffler's method.

Pneumococcus. Cases of membranous inflammation of the fauces, occurring during the course of pneumonia, have frequently been observed, and many instances have also been recorded unaccompanied by pneumonia [Concetti (1893), Chaillou and Martin (1894), De Blasi and Russo-Travali (1896), Möller (1903), etc.]. Some examples of very extensive lesions are recorded, for example exudation covering the tonsils, pillars of the fauces and uvula (Jaccoud, 1891), or the entire pharynx, palate, lips, and nasal cavities (Vedel, 1898). In both these instances pneumococci were the only organisms found, and in the latter case their virulence was proved. Perhaps the most remarkable example of extensive membrane formation by the pneumococcus is that described by Cary and Lyon (1901). During an attack of acute lobar pneumonia there occurred profuse pseudo-membranous exudations upon nearly all the mucous membranes of the body, tonsils, lips, gums, cheek, under surface of tongue, soft palate, fauces, nose, conjunctiva, anus, and glans penis. Diphtheria bacilli, streptococci, saccharomyces, etc., were all looked for, but were not found. Virulent pneumococci were proved to be the only organisms present in all the lesions. Another very interesting case was observed by Netter (1891). A boy aged three years suffering from varicella showed urgent symptoms of laryngitis requiring tracheotomy, through the wound of which a false membrane was expelled containing no diphtheria bacilli but only pneumococci. A similar case is also recorded by Seuvre (1898). In summarising his observations on the infections of the mucous membranes by pneumococcus Foulerton (1902, p. 293) makes the following remarks. "The exact bacteriological identification is rendered difficult by the fact that the organism is a frequent parasite of the healthy mouth. But the number of cases in which there are the formation of false membrane and destruction of the superficial layer of the epithelial membrane associated with the presence of the coccus and without any other of the bacteria —*B. diphtheriae*, *Streptococcus pyogenes*, *Saccharomyces albicans*— which are known to cause similar lesions, leaves little doubt as to the fairly frequent occurrence of an acute membranous pharyngitis due to this cause."

Bacillus of Friedländer. This organism has been occasionally found in cases resembling diphtheria (Michelazzi, 1904). Nicolle and Herbert (1897) found it in pure culture in six out of 1,600 cases of angina, and state that it is capable of producing chronic, subacute and acute anginas, sometimes accompanied by a continuous membrane.

Bacillus coli. Lemoine (1895) observed bacillus coli together with streptococci and pneumococci in false membranes, and Chaillou and Martin (1894) noted two cases in which liquefying organisms resembling bacillus coli were alone present.

Fusiform bacilli. Vincent (1896) was one of the first to call attention to the presence of fusiform bacilli and spirilla (Pl. XIV, fig. 4) in connection with ulcerative and membranous conditions in the mouth.

Consequently the pathological process associated with these organisms is frequently termed Vincent's angina. Since 1896 numerous observers[1] have noted their presence in various lesions, and even in the healthy mouth. These fusiform bacilli are of considerable interest since many of the conditions in which they are found closely simulate diphtheria, and the organisms themselves have to be carefully distinguished from diphtheria bacilli in preparations made directly from the throat. Although they have been so frequently detected in such preparations their isolation has been attended with difficulty, and appears only to have been successfully accomplished by three investigators, Veillon and Zuber (1898), Ellerman (1904) and Weaver and Tunnicliffe (1905). The following description is taken from the latter authors:

In smear preparations the bacilli are seen as long slender rods usually with pointed ends. Occasionally the ends are rounded, or the rod thicker, or it is slightly bent. Their length varies from 6—12 μ. They are generally scattered uniformly over the field but sometimes irregular clumps are seen. They may be stained by Loeffler's methylene blue, but are best stained with carbol fuchsin, and do not retain Gram's stain. They are non-motile and show no polar bodies. The bacilli may be grown in mixed culture in sugar-free broth, with or without the addition of ascites fluid. Pure cultures can be obtained by smearing the surface of horse-serum-agar slants and cultivating anaerobically at 37° C. for 3—5 days.

In pure culture the organisms are obligatory anaerobes. The colonies appear as delicate white discs which become confluent. A small moist growth is produced on Loeffler's serum, a delicate white confluent growth on ascites agar, a delicate cloud along the line of inoculation on agar, and a slight flocculent growth in sugar-free broth (Pl. XIV, fig. 5). There is no growth in milk, nutrient broth, or on potato. Cultures on media containing serum or ascites fluid give off a very foul odour.

On ascites agar, horse-serum-agar and Loeffler's medium they no longer appear as fusiform bacilli but as long delicate filaments with darker bodies at intervals (Pl. XIV, fig. 6). Veillon and Zuber (1898) produced small abscesses in rabbits and guinea-pigs by the inoculation of pure cultures, but Weaver and Tunnicliffe (1905) found that injections of pure cultures into the muscles or under the skin of rabbits and guinea-pigs were without result. In mixed cultures they produced abscesses.

Tunnicliffe's (1906) most recent investigations point to the conclusion that the fusiform bacilli and spirilla are not different organisms but merely different stages in the life history of the same organism. She describes the various appearances met with in cultures in the following words: "The organisms present the same morphological appearance in whatever media grown. They are extremely polymorphous, appearing as quite different organisms at different periods of their development. They are usually during the first 24 hours of their growth delicate pointed rods from 3 μ to 10 μ in length. As a rule they show deeply staining bodies or bands, most often two in number, not situated at the ends. The bacilli are usually straight, sometimes bent. The bacilli often strikingly resemble the barred forms of diphtheria bacilli. They are often slightly larger in the centre but not always. In the young cultures a few smaller, 1·5—4 μ in length, and plumper bacilli are also sometimes found. They have very thick unstained bodies, with deeply stained rounded ends. The swollen bodies resemble spores, but do not stain as such. Both forms appear in pairs, end to end, at obtuse angles, and in rows. In some of the longer bacilli, usually during the first days of their growth, a few spores are seen. There is usually one in a bacillus, but occasionally there are two. They are situated either at one extremity or near the centre. They may be seen within, or partly without, or entirely outside the bacillus. The development from the round spores into the very short plump bacilli with dark extremities may be observed in a hanging block. In 24 hours or even later, filaments of various lengths are formed. Some of these filaments are of the same diameter throughout and contain as a rule deeply staining bodies, sometimes round, oftener like bands. Similar ribbon shaped forms are frequently seen in smear preparations from the gums. Some of the filaments stain uniformly. As a rule many of the filaments are seen to be made up of strings of bacilli, which are joined together at the dark bodies. The bacilli forming the filaments vary in size and shape as the bacilli in the earlier cultures do. The filaments are sometimes straight, sometimes wavy. Involution forms in a great variety of shapes are frequently observed. In the older cultures the filaments often stain irregularly. Clear spaces resembling vacuoles are often seen. They are simply the unstained bodies of the short, plump bacilli which in chains are so close together as to appear as vacuoles.

---

[1] Beitzke (1904) gives an excellent *résumé* of the literature.

"Soon after or simultaneous with the appearance of the filaments, most often on the fourth or fifth day, spirals are observed, sometimes in enormous numbers. As a rule they stain uniformly; others show the dark bodies seen in the short bacilli and filaments. Often it is easily seen that the spirilla are made up of chains of short bacilli similar to the straight filaments. The spirals are sometimes in the form of corkscrews, more often the turns are not so sharp, nor so deep. They form one to twenty curves. The turns are sometimes rounded, sometimes very pointed. The pointed ones are especially marked when the spirilla can be seen to be made up of bacilli and when there is only one bend. Some of the larger spirals extend across the whole field, more often they are shorter, showing four or five turns. These shorter forms are from 4—5 μ in length. They vary considerably in the depth of the curve as do the uncultivated spirilla. The ends are usually pointed. Toward the extremities the curves sometimes become more and more broad. Involution forms are seen in the spirilla as well as in the filaments. In some of the cultures the spirilla alone are found, but usually the filaments and short bacilli are also present. By the 10th to the 15th day fewer filaments and spirilla are seen, but as a rule, even in the older cultures (55 days), spirilla can still be found. Both the bacilli and spirilla stain by methylene blue, gentian-violet, Giemsa, Romanowsky, carbol-fuchsin, and carbol-gentian violet."

Cultures of all ages inoculated into guinea-pigs produce no result.

Leptothrix. Meunier and Bertherand (1898) describe a case in which a diphtheria-like membrane was found. A leptothrix was present in great abundance on the tonsils, uvula, and soft palate. Sections of the membrane showed large numbers of these organisms.

Yeasts. Pseudo-membranous lesions are occasionally met with, in which yeasts, usually *Saccharomyces albicans*, are the most abundant, or the only organisms to be found.

Syphilis. Campbell (1904) and others have called attention to the resemblance to diphtheria of chancre of the tonsil. The chancre itself may have a pseudo-membranous appearance and the accompanying general phenomena and enlarged glands add to the difficulty of diagnosis. Even an intranasal chancre has been mistaken for nasal diphtheria (Rolleston, 1906).

CHAPTER XI.

PREVENTIVE MEASURES.

General preventive measures. Notification. Isolation. Disinfection. Source of infection. Closure of schools. *Special preventive measures.* Primary and secondary examinations of clinical cases and contacts. Classes of persons who ought to be examined. Isolation of mild cases and infected contacts. Infected families. Schools. Isolation homes. Hospitals and institutions. Period of isolation. Causes of misleading negative cultures. Proportion of negative followed by positive cultures. Persistence of diphtheria bacilli in the throat and nose. Attempts to hasten the disappearance of the bacilli. Results of isolation of convalescents, mild cases, and contacts in families, schools, institutions, hospital wards, and towns during outbreaks. The prophylactic injection of antitoxin. Summary.

Preventive Measures.

THE measures which may be taken to check the spread of diphtheria will be most conveniently discussed under two headings, namely (1) general and (2) special measures. Under the heading of general preventive measures are considered those means of prevention which are applicable in the case of any other severe infectious disease, and the special modifications which are necesssary in the case of diphtheria, whether they apply to the individual, or to collections of individuals, or food substances. The application of bacteriology in checking the spread of the disease will be discussed under special preventive measures.

I. General Preventive Measures.

Notification. Persons suffering from diphtheria are required by law to be notified to the Medical Officer of Health. In the case of persons showing the typical symptoms of diphtheria no difficulty arises. The fact that the disease may manifest itself in a variety of ways and that during outbreaks many persons may be encountered who are carrying virulent bacilli, without any signs of ill-health, complicates the question of notification. Whether persons suffering from atypical forms of the disease and healthy infected contacts should be notified are points which have frequently been discussed. Most authorities agree that the former class should be notified as suffering from diphtheria, and treated in the same manner as those who show typical clinical symptoms. On the other hand the general opinion is that healthy infected contacts should not be notified, but should be dealt with separately from those who show clinical signs. A few authorities, however, consider that even these persons ought to be notified. This question was very carefully considered by Cobbett (1901) in dealing with his first outbreak at Cambridge, and he came to the conclusion that the notification of such persons was undesirable for many reasons.

Diphtheria is a disease, and therefore a person cannot be held to have diphtheria who remains perfectly well, and notification is resented on this ground.

"It has been pointed out that without notification the Medical Officer of Health has no power to deal with these persons, but that armed with this instrument he can compel them to be isolated. In answer to this, it may be said that he could only compel the removal of those for whom it could be shown that isolation was impossible at home, and that too on the receipt of an order from a magistrate." Further, compulsory isolation following on notification would raise general opposition to all measures attempting to deal with these persons, and since persuasion has in most cases been perfectly satisfactory it is undesirable to create opposition by resorting to forcible measures.

The fact is that nothing can be done unless the people back up the measures. When it is a case of dealing with public schools, and the class of persons who send their sons to them, there is little or no difficulty in carrying out the bacteriological examination of contacts, and the parents see to the isolation of their infected children, for they at once recognise that the measures proposed are for their own interest. The poorer class will take the same view if the matter is fairly explained to them. We must therefore in such matters act by persuasion rather than by force. If persuasion fails, the education authorities have the power of refusing to allow the infected scholars to return to school until they have been officially certified to be free from diphtheria bacilli.

Isolation. Isolation hospitals[1] are provided by the Local Authorities in which patients suffering from diphtheria, who cannot be efficiently isolated in their own homes, may be treated. In whatever way patients are isolated it is highly desirable that the isolation should be efficiently carried out so that the possibility of the spread of the disease by contact should be reduced to a minimum. In all cases the methods by which the disease is conveyed from person to person should be explained to the attendants, and precautions taken to prevent its dissemination by infected objects.

Disinfection. The various examples which have been quoted of the finding of diphtheria bacilli on infected articles and in dust show the necessity for sterilising, whenever possible, all articles and toys which have been used by the patients immediately before and after the development of clinical signs. Although the probability of infection by means of dust, etc., is very small, it is generally customary to disinfect the room occupied by the patient after removal to the hospital, or after the termination of isolation, if the patient has been kept at home.

During the progress of the disease all cups, plates, etc., used by the patient ought to be sterilised by boiling after use, and all soiled linen and other similar articles ought to be sterilised by some efficient means. Articles of little value and dressings should be burnt.

The importance of these precautions, which relate more particularly to the spread of the disease to family or very close contacts, is widely acknowledged, and once the disease has been recognised more or less efficient precautionary measures are usually adopted.

Comparatively little attention has, however, been paid to similar precautions for checking the spread of diphtheria bacilli amongst school children. It has already been shown that the bacilli are most frequently communicated by means of articles such as pencils, which pass from mouth to mouth. Suggestions have frequently been made, that each child should possess its own slate, pens, pencils, etc., and that these articles should be frequently and systematically sterilised when diphtheria is prevalent. In institutions where the children receive one or all their meals, the same precautions ought to be taken in regard to knives, forks, cups, etc.

Source of infection. Once diphtheria has broken out it becomes the duty of the authorities to trace as far as possible the origin of the infection, so as to check any further spread from that source.

---

[1] The provision of Isolation Homes for infected contacts belongs more appropriately to the Bacteriological measures and is discussed later (p. 405).

Since the source of most infections is personal contact, inquiries ought to be first directed to the possibility of infection having occurred in this way, and a bacteriological examination made of the members of the patient's family and any persons whom the inquiry may indicate to have been possibly instrumental in carrying the disease. By this means it is frequently possible to detect mild unrecognised cases which act as carriers (p. 309). Bacteriological examinations ought also to be made of persons who are likely to have been infected by the patient, and may be capable of transmitting the disease. The classes of persons whom it is desirable to examine under these conditions are fully considered later (p. 401).

If no source of personal contact is discovered, and especially if cases arise apparently independently of one another, suspicion is directed to the milk supply. Here again the bacteriological examination of those who are connected with the cows, the dairy, and the distribution of the milk is likely to be of more service than the examination of the milk itself, since the isolation of the diphtheria bacillus from milk is a difficult matter, and has only been successfully accomplished on three occasions. Still more rarely has it been definitely proved that the cows themselves are the source of the disease (p. 285). Suspicion has frequently been directed to other domestic animals and birds as the source of the disease, but bacteriologically the suspicion has very seldom been confirmed (p. 304). Infected articles used by many persons, such as speaking tubes, and the cups of public fountains, etc., may occasionally play some part in the dissemination of the disease, and ought not to be neglected. The list, which has been given, practically exhausts all the means by which the disease has been proved to have been propagated, since no trustworthy evidence exists that it may arise through the agency of putrefying refuse, sewer gas, polluted soil, or unhealthy surroundings. By their effect on the general health these agencies may, however, render those who are exposed to their influence more susceptible to the disease.

Schools. The influence of schools and institutions, in which large numbers of individuals of susceptible age are brought together, is a subject which has received great attention. It seems to be now generally held that such places play an important part in the dissemination of the disease through the presence of mild unrecognised cases and infected contacts, who readily pass on the bacilli which they have acquired owing to the habits which prevail amongst the children (p. 318).

In serious outbreaks it has generally been the custom to close the schools with the object of preventing the daily meeting of large numbers of children. No doubt to some extent this proceeding succeeds in its object, but it has the disadvantage of rendering the examination of the scholars more difficult, and the probability of discovering most of the carriers of the bacilli more remote. Further, while the children are attending the schools cases of mild sore throat and other ailments are brought to the notice of the authorities and can be immediately examined, whereas while the children are at home such slight ailments are liable to pass unnoticed. If such cases are found on bacteriological examination to be suffering from mild diphtheria, their immediate contacts can be easily singled out for examination. It is therefore desirable, whenever possible, to bacteriologically examine the school children before the closing of the schools, and thereby take the opportunity of dealing with the infected children who may be discovered. The methods which have been employed in dealing with these persons and their results are given in the next section. The question of dealing with the public elementary schools is complicated by the fact that the children from the infected schools are liable to mix on Sundays with the children from non-infected schools in the Sunday Schools. This intermingling of the scholars from healthy and infected day schools is probably a frequent cause of the extension of the disease to schools which up to that time have remained uninfected. The closing therefore of Sunday Schools is one of the first measures which ought to be enforced when diphtheria becomes prevalent.

II. Special Preventive Measures depending on Bacteriological Methods.

More is definitely known about the transmission of diphtheritic infection than of any other epidemic disease. Owing to this fact and the ease with which most suspected exudates and materials can be examined, diphtheria readily lends itself to bacteriological methods of prevention. In this section the various bacteriological preventive measures which have been used, and the measures which depend on them, together with the results which have been obtained, are discussed.

The bacteriological measures may be considered under the following headings:—

(1) The primary examinations of clinical cases and the subsequent examinations of convalescents. (2) The primary examinations of contacts

and the subsequent examinations of those who are found to be infected. (3) The classes of contacts who should be examined in connection with cases of diphtheria. (4) The isolation of infected contacts. (5) The time during which convalescents and infected contacts should be isolated. (6) Records relating to the persistence of diphtheria bacilli in the throat and nose, etc. (7) Attempts to hasten the disappearance of the bacilli. (8) The results of isolating convalescents and infected contacts until the disappearance of the diphtheria bacilli in (*a*) families, (*b*) schools, (*c*) institutions and hospital wards, and (*d*) towns. (9) The prophylactic injection of antitoxin.

(1) *The primary examinations of clinical cases and the subsequent examinations of convalescents, and* (2) *the primary examinations of contacts and subsequent examinations of those who are found to be infected.*

The methods of practical bacteriological examination in all these cases have already been discussed (p. 340) at some length, but a statement of the principal points may be desirable. The bacteriological diagnosis in most clinical cases is easy, but in all instances in which doubtful bacilli are found it is the duty of the bacteriologist to test and prove the correctness of his opinions by every means in his power. When the patient is becoming convalescent the bacilli may be present in large numbers or their numbers may be very small. In the latter case especial care should be taken to obtain satisfactory swabs, at least 12 hours after the application of any antiseptic substances.

Should no diphtheria bacilli be encountered at the first examination the cultures should be recultivated for a period of 24 hours and again examined. When the bacilli has disappeared from the seat of the original lesion, the other situations in which they are liable to lurk should also be examined, in order that the patient should not return to the community still harbouring virulent diphtheria bacilli.

In the case of infected contacts the bacilli may or may not be abundant from the start. It is therefore desirable in examining contacts to subject all negative cultures to a further period of incubation and examination. The same precaution ought to be observed in all the subsequent examinations of infected contacts.

(3) *The classes of persons who ought to be examined in connection with cases of diphtheria.*

Reference to the section on Infection by Contact shows that the proportion of infected contacts is highest amongst the members of the family of the patient, next highest amongst close contacts in hospital wards and institutions and lowest amongst the more remote contacts in schools. A careful examination of the results of the observations on wards, institutions and schools reveals the fact that in these cases the proportion of infected contacts is low, because many are examined who have had no direct relationship with the case. It is those who have been in attendance on the patient, or have been situated near him either in wards or in schools, who are most commonly found to be infected. Illustrations of these facts have already been given (pp. 309—318).

It therefore follows that in connection with a case of diphtheria the members of the family, and all who have been in close connection with him at home, should be immediately examined, as well as his more intimate friends, and at least certain members of his class who sat near him at school. These proceedings not infrequently reveal mild unrecognised cases and healthy infected contacts, especially in connection with those instances in which the clinical signs are at first mild, and only after some days become sufficiently severe to be recognised. The discovery of unrecognised cases has not only frequently led to the prevention of further transmission of the disease, but has afforded valuable information as to its origin.

When a serious outbreak has to be dealt with in a school, it is no longer possible to hope that these measures will be sufficient to trace all the infected persons. It then becomes desirable to examine all the members of the class to which patients belong, or even the whole school.

When an outbreak of diphtheria occurs in a hospital ward, the throats and noses of all attendants as well as of the occupants of the ward, especially those who are closely connected with the patients either by occupying adjacent beds or by visiting them, should be examined. In epidemic times outbreaks of diphtheria in the wards of general hospitals may be prevented by systematically examining all the children who are admitted.

The same procedure has met with considerable success in preventing the introduction of diphtheria into Scarlet Fever wards (Chapter XII). In all these cases special attention should be directed to persons who show any abnormal condition of the fauces or nose, or who are suffering from ear discharges.

When there is reason to suspect the milk supply as the cause of the outbreak not only should the same precautions be taken as in other outbreaks, but a thorough examination should be made of all persons who are connected with the milking of the cows, and with the dairy, and their families. When the distribution of the disease points to the infection of the milk during delivery, it would also be desirable to examine any persons who had access to the milk after it left the dairy along the infected route.

(4) *The isolation of mild cases and infected contacts.*

Almost all authorities have advocated the isolation of persons, who are found to be suffering from abnormal conditions of the throat or nose associated with the presence of diphtheria bacilli. On the other hand while some have advocated the isolation, whenever possible, of all infected contacts, even those who show no manifestations of disease, others think, that while it is theoretically desirable to do so, this proceeding is impracticable except in those schools in which the scholars reside on the premises, hospital wards and certain institutions. Others again appear to believe that the danger of infection from such persons is so slight that measures to insure their isolation are, in the majority of cases, unnecessary. Some of those who take this latter view are not averse to the isolation of infected contacts, when isolation can be easily accomplished.

Reference to the beneficial results which have followed on the isolation of healthy infected contacts under all conditions, indicates the desirability of isolating infected contacts, whenever this can be accomplished (see p. 424).

Infected Families.

When an infected contact is discovered amongst the children of an infected family belonging to the well-to-do class, it is frequently sufficient to explain to the parents the danger which the other members of the family run from the presence of this child amongst them. Arrangements can then usually be made for the isolation of this child in some room, until the bacilli have disappeared, and for the adequate disinfection of the articles used by him. A person who is not in the habit of mingling with the healthy members of the family should, if possible, attend on the infected child. Amongst the poorer classes such an arrangement is usually impossible both from lack of accommodation and from the fact that no one can usually be spared to see that efficient isolation is maintained. It is also generally extremely difficult to make the parents understand that all danger has not passed within a few days.

Consequently in such cases home isolation is a mere farce, and an attempt should be made to obtain the removal of the child to an isolation home, or some place where it can not only be kept from contact with other susceptible individuals, but treated with antiseptics in order if possible to hasten the disappearance of the bacilli.

A more serious question arises when it is found that one of the adult members of the family is harbouring diphtheria bacilli. In the well-to-do classes there is little difficulty in making some satisfactory arrangement whereby the probability of the younger susceptible members becoming infected is very greatly diminished. Amongst the poorer classes, however, more difficulty is experienced in deciding how to treat these persons. On the one hand the infected person may be a source of danger to other adults under certain conditions. There is, for example, a grave risk of spreading the disease when the infected person is employed in a factory, shop, or other place of business where the employees are constantly in close contact or have their meals together. On the other hand the children of the family are liable to become infected from this individual, and convey the disease to other children. Under these circumstances the employer is sometimes willing to allow such a person to stay away until he is free from diphtheria bacilli. Unless an arrangement of this description can be made, the infected individual cannot be compulsorily isolated and the family deprived of their means of livelihood. Cobbett (1901, footnote, p. 488) met with this difficulty and decided that "it was not expedient, as a rule, to examine the parents or bread winners, on account of the impossibility of isolating them without provision being made for the support of those dependent on them."

Wesbrook (1900) had previously suggested in his regulations for quarantine in diphtheria that wage-earning members of the families should be exempted from isolation as long as they did not remain in contact with the infected members.

If it is decided that it is not desirable to examine parents and bread-winners, the risk of some of them being infected and spreading the disease has to be borne in mind in investigating the source of infection in fresh cases. Two instances illustrating the conveyance of the disease by infected parents have recently come under the writer's notice. In each instance one of the children developed clinical diphtheria, and the other children of the family were examined and were found to be free from diphtheria bacilli. In one family two cases and in the other one case arose between four and five weeks later. Again on examination the other children were found to be free, but in one family the mother, and in the other the father, were found to be harbouring virulent diphtheria bacilli. The former had also given the infection to a neighbour's child, which she had nursed when it was suffering from the effects of a slight accident.

Schools.

In schools, whether large or small, attended by the children of the well-to-do classes the isolation of mild cases and infected contacts can generally be easily arranged in some of the buildings or rooms of these establishments.

The isolation of such children when found among the scholars of the public elementary schools is, however, a more difficult matter. As has been pointed out the mere exclusion of these children from the schools and the attempt to isolate them efficiently in their homes are somewhat unsatisfactory, although considerable benefit has been derived from these measures in some instances. It also seldom happens that there are any suitable rooms or buildings connected with such schools which can be used for the purpose. If, therefore, any attempt is made to check the spread of the disease through infected contacts, suitable isolation homes ought to be provided for them. In epidemic times a suitable house in some more or less isolated position can usually be procured and used as an Isolation Home under the direction of properly qualified nurses. Such Isolation Homes have been provided at Cambridge (1901–4) and Colchester (1901).

Isolation Homes.

In every instance in which any form of Isolation Home has been used both healthy infected contacts and mild cases of diphtheria have been admitted at the same time.

This association of healthy persons with cases of diphtheria cannot be otherwise than dangerous, unless the bacteriologist can be relied upon to exclude all but those who are harbouring the true diphtheria bacillus. Healthy individuals harbouring the virulent diphtheria bacillus are not likely to be harmed by receiving diphtheria bacilli from others. This argument does not, however, necessarily apply to those persons who are found to be harbouring the non-virulent diphtheria bacillus. It may be questioned whether it is necessary to isolate these persons, and whether, if isolated amongst those who carry about the virulent diphtheria bacillus, they are liable to catch diphtheria. In practice, however, the virulence of the bacillus is only determined after isolation has been carried out. The probability of the interchange of bacilli amongst the isolated persons may be almost completely abolished by the frequent use of antiseptics, and by setting apart particular knives, cups, etc. which can be frequently sterilised, for the use of each inmate.

Under these conditions the experiences of Cobbett (1901) and Graham-Smith (1902–4) show that no ill effects are produced by the isolation of all these classes of persons in one place. The former (p. 491) records some very interesting observations on this subject. Five children, harbouring non-virulent diphtheria bacilli, lived in close contact for some time in a Home with twelve others, who were infected with diphtheria bacilli known to be virulent in the case of eight. This action was followed by no bad results, no case of diphtheria or even sore throat occurring amongst the healthy persons in the Home.

"From seven of the persons isolated in this Home, the bacilli were twice or oftener isolated and tested for virulence on the guinea-pig. The result was striking. Those admitted with a non-virulent diphtheria bacillus were never found to have acquired a virulent bacillus during their stay in the Home, nor was a non-virulent diphtheria bacillus ever found in a child in whom virulent bacilli had once been found. In the case of E. J. the bacilli (non-virulent) were isolated and tested 10 times in the course of the 15 weeks she remained in the Home. During five of these weeks her little sister V. J. was with her constantly and on three occasions the bacilli were isolated from her and proved fully virulent. Moreover from another girl, G. B., who remained in the Home almost as long as E. J., diphtheria bacilli were isolated and proved virulent no less than six times." The same evidence, but less strong, is afforded by the rest of the seven cases mentioned above.

Hospitals and Institutions.

Hospitals, Asylums and various Public Institutions generally afford ample facilities for the isolation of infected persons.

(5) *The period during which isolation of convalescents and infected contacts ought to be maintained.*

Even up to the present time very diverse views are held as to the time during which convalescents ought to be isolated. Judging from certain reports there are some local authorities who allow diphtheria patients to return to their homes almost as soon as the clinical manifestations of the disease have disappeared, while others fix an arbitrary time limit from the date of the disappearance of the last symptoms. The time most commonly chosen is about four weeks. It is, however, now becoming very generally recognised, that such arbitrary time limits, although they may cover the period of infectiousness of the majority of patients, allow the return to the community of many individuals who are still capable of transmitting the disease.

The only means by which the patients can be proved to be no longer dangerous is the bacteriological test. The period of isolation or quarantine should therefore in all cases be regulated by the bacteriological findings. Amongst those who advocate this means of regulating the period of isolation, differences of opinion still exist as to the significance of the organisms encountered and the importance which should be attached to them. While some consider that isolation should be continued until the disappearance of the morphologically typical diphtheria bacilli, others think that even the typical bacilli lose their virulence during a prolonged stay in the throat or nose, and that after a time their presence in small numbers is no longer of much importance. Numerous investigations have already been quoted (p. 236) which show that diphtheria bacilli may retain full virulence for very long periods, and consequently without animal experiments there is no ground in any given case for considering that their virulence has diminished and that their presence may be neglected. Others again hold that diphtheria bacilli during convalescence become converted into Hofmann's bacilli, which are capable under unknown conditions of regaining their pathogenicity.

If the views of the latter group were followed patients would have to be isolated until the disappearance of all diphtheria-like bacilli. Figures, which have been already quoted (p. 205), show how commonly Hofmann's bacilli are found in the secretions of normal persons, and reference to many cases given in the following tables (pp. 408—413) shows that these organisms may persist for an indefinite time in the mouths of convalescents and healthy contacts.

Further it has been shown that there are many reasons for believing that the Hofmann's bacillus and other diphtheroid organisms are distinct species, having no connection with the diphtheria bacillus (Chapter VI). It is, moreover, impracticable to isolate many individuals until the disappearance of such organisms. In practice, therefore, this method cannot be adopted.

Table showing the virulence of diphtheria bacilli isolated from 112 *persons, clinical cases and contacts, out of* 115 *persons found to be harbouring diphtheria bacilli during an epidemic at Cambridge* (*Graham-Smith*, 1904).

(a) = Notified case. Diphtheria bacilli found.
(b) = "Contact" infected with diphtheria bacilli.
Δ = Virulent diphtheria bacillus.
Λ = Non-virulent diphtheria bacillus.
O = Diphtheria bacillus not found.
* = Hofmann's pseudo-diphtheria bacillus found.
SS = Small swelling in subcutaneous tissue of guinea-pig.
MS = Moderate " " " "
LS = Large " " " "
+ = Death of guinea-pig.
} In I, II, III or IV days, *i.e.* within 24, 48, 72 or 96 hours.

N = No result. Guinea-pig not affected.
dark red = Suprarenals very dark in colour.
red = Suprarenals deep red in colour.
pink = Suprarenals pink in colour.
present = Pseudo-diphtheria bacilli found on one or more examinations.
0 = " " " not found at any " (last column).

No.	Initials	Results of consecutive examinations	Days during which diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations						Results of Autopsy			Pseudo-diphtheria bacillus
					Wt. of guinea-pig in grammes	Dose of 48 hours broth culture in c.c.	Results. Days				Oedema	Supra-renals	Fluid in pleura	
							I	II	III	IV				
St Matthew's School (Girls)														
1	A.S. (a)	$0.0.\Delta.\Delta.\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{0}$	72	acid	170	·2	MS	+			{extensive haemorrhagic	dark red	trace	present
2	W.B. (a)	$\Delta.0.0.0$	8	"	170	·2	SS	+			extensive	red	-	0
3	G.G. (a)	Fatal. No examinations												
4	K.M. (b)	$\Lambda.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	8	acid	{180	·2	N	N	N	14th day N	-	-	-	present
					{200	2·0	N	N	N	N	-	-	-	
5	G.C. (b)	{$\Delta.\Delta.\Delta.\Delta.0.\Delta.0.\Delta.\Delta.\Delta.\Delta.0.0.$ {$0.\Delta.0.0.0$	44	"	240	·2	SS	+			slight	dark red	-	0
6	W.L. (b)	$\Delta.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	8	"	210	·1	LS	+			extensive	"	·8 c.c.	present
7	E.P. (b)	$\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	8	"	235	·1	MS	+			"	"	3·2 c.c.	present
8	C.S. (b)	$\underset{*}{\Delta}.\Delta.\Delta.\underset{*}{\Delta}.\underset{*}{\Delta}.\Delta.\Delta.\Delta.\Delta.0.\underset{*}{0}.0$	29	"	180	·2	MS	LS	+		very extensive	"	-	present
9	R.S. (b)	$\Delta.\Delta.\Delta.0.\Delta.0.0.\Delta.0.0$	83	"	280	·1	MS	+			{extensive haemorrhagic	"	trace	0
St Matthew's School (Infants)														
10	H.O. (a)	$\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	21	"	not tested						-	-	-	present
11	F.A. (a)	$\underset{*}{\Delta}.\underset{*}{0}.0.\underset{*}{0}$	17	"	230	·1	MS	+			extensive	dark red	4·6 c.c.	present
12	F.E. (a)	$\underset{*}{\Delta}.0.\Delta.\underset{*}{0}.\Delta.0.0.0.0.0$	68	"	160	·2	SS	+			{extensive haemorrhagic	"	-	present
13	F.H. (a)	Δ. Fatal	-	"	170	·2	MS	LS	+		extensive	"	-	-

14	M.F. (a)	$\Delta.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	28	acid	190	·2	MS	+			extensive	dark red	·9 c.c.	present
15	N.W. (a)	$\underset{*}{\Delta}.\Delta.\Delta.\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	24	,,	430	·3	SS	+			extensive haemorrhagic	,,	-	present
16	D.H. (b)	$\Delta.\underset{*}{\Delta}.\Delta.\underset{*}{0}.0.\Delta.\underset{*}{\Delta}.0.\underset{*}{0}.\underset{*}{0}.0$	29	,,	225	·1	MS	LS	LS	+	,,	red	3·4 c.c.	present
17	D.E. (b)	$\Delta.\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}.0.0$	14	,,	225	·1	SS	+			,,	,,	trace	present
18	V.L. (b)	$\underset{*}{\Delta}.0.\underset{*}{0}.\Delta.\Delta.\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{0}.\Delta.\Delta.\Delta.0.0.0$	41	,,	270	·1	MS	LS	+		,,	dark red	·4 c.c.	present
19	M.P. (b)	$\Delta.\Delta.\underset{*}{\Delta}.\Delta.0.\underset{*}{0}.\underset{*}{0}$	20	,,	240	·1	SS	+			extensive	,,	1·0 c.c.	present
20	T.E. (b)	$\Delta.\Delta.\Delta.\Delta.0.0.0$	22	,,	200	·1	SS	SS	+		slight	red	-	0
21	H.W. (b)	$\Lambda.\Lambda.\Lambda.\underset{*}{\Lambda}.\Lambda.\Lambda.\Lambda.\underset{*}{0}.\Lambda.0.0.\Lambda.\Lambda.$ $\underset{*}{\Lambda}.\underset{*}{0}.\underset{*}{\Lambda}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	52	,,	200 †200	·2 2·0	N SS	N N	N N	14th day N N	- -	- -	- -	present
22	B.T. (b)	$\underset{*}{\Delta}.0.\underset{*}{0}.\underset{*}{0}$	9	,,	235	·2	LS	+			extensive haemorrhagic	dark red	2·3 c.c.	present
23	S.C. (b)	$\Delta.\underset{*}{\Delta}.\Delta.\underset{*}{\Delta}.\Delta.\Delta.\underset{*}{\Delta}.\underset{*}{0}.\Delta.\underset{*}{0}.\underset{*}{\Delta}.\underset{*}{\Delta}.0.$ $\underset{*}{0}.\Delta.0.\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	51	,,	300	·1	MS	LS	LS	+	extensive	,,	-	present
24	G.T. (a)	$\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	17	,,	150	·2	MS	+			extensive haemorrhagic	red	-	present
25	S.M. (b)	$\Delta.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	9	,,	150	·1	SS	+			extensive	dark red	-	present
26	F.S. (b)	$\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.0.\Lambda.0.$ $\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.0.\Lambda.\Lambda.0.\Lambda.\Lambda.$ $\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.$ $\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.\Lambda.0.\Lambda$	122	,,	180 ‡210	·2 2·0	N N	N N	N N	14th day N N	- -	- -	- -	0
27	W.O. (b)	$\underset{*}{\Lambda}.\underset{*}{0}.\underset{*}{0}.0$	9	,,	200	·2	N	N	N	14th day N	-	-	-	present
28	A.M. (b)	Λ	-	,,	340	2·0	N	N	N	14th day N	-	-	-	-
29	L.R. (a)	$\underset{*}{0}.\Delta.\underset{*}{\Delta}.\underset{*}{0}.0.\underset{*}{0}$	30	,,	170	·2	MS	+			slight	dark red	-	present
30	M.H (a)	$\Delta.$ Fatal	-	,,	410	·2	SS	MS	+		extensive haemorrhagic	,,	2·5 c.c.	-
31	J.M. (a)	$\Delta.\Delta.\Delta.\Delta.\Delta.\Delta.\Delta.0.0.\Delta.0.\Delta.0.$ $0.0.0.0.0.\Delta.0.0$	58	,,	210	·2	SS	+			moderate	,,	-	0
32	A.M. (b)	$\underset{*}{\Delta}.\Delta.\underset{*}{0}.\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{\Delta}.\Delta.\Delta.\Delta.$ $\Delta.\underset{*}{0}.\Delta.\Delta.\underset{*}{0}.\Delta$	47	,,	250	·2	SS	+			extensive	,,	12·0 c.c.	present
33	W.R. (a)	$\Delta.\underset{*}{0}.\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	32	,,	225	·2	LS	+			,,	,,	1·0 c.c.	present

† Bacilli from 7th examination.

‡ Bacilli from 28th examination.

No.	Initials	Results of consecutive examinations	Days during which diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations						Results of Autopsy			Pseudo-diphtheria bacillus
					Wt. of guinea-pig in grammes	Dose of 48 hours broth culture in c.c.	Results. Days I	II	III	IV	Oedema	Supra-renals	Fluid in pleura	
34	C.L. (a)	Δ. Fatal	-	acid	195	·2	MS	+			extensive	dark red	3·2 c.c.	-
35	F.B. (a)	Δ.0.Δ.Δ.Δ.Δ.0.0.0.0.0	27	,,	210	·2	SS	MS	+		,,	,,	-	0
36	A.C. (a)	Δ. Fatal	-	,,	220	·2	MS	+			,,	red	6·3 c.c.	-
37	R.G. (a)	Δ.Δ.Δ.0.0.0.0.0.	25	,,	150	·2	SS	+			,,	dark red	2·0 c.c.	0
38	J.G. (a)	Δ.0.0.0.0.0	6	,,	190	·2	LS	+			,,	,,	4·0 c.c.	0
Sturton Street School														
39	A.L. (a)	$\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	14	,,	260	·1	SS	MS	+		,,	,,	1·3 c.c.	present
40	G.S. (b)	$\underset{*}{\Delta}.\Delta.\underset{*}{\Delta}.\underset{*}{0}.\Delta.\Delta.\Delta.\Delta.\underset{*}{\Delta}.\Delta.\Delta.\Delta.\Delta.$ $\Delta.\Delta.\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}.\Delta.\underset{*}{\Delta}.\underset{*}{0}.\Delta.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	27	,,	260	·3	LS	LS	+		extensive haemorrhagic	,,	6·3 c.c.	present
41	J.T. (a)	$\Delta.\Delta.\Delta.0.\underset{*}{\Delta}.\Delta.\underset{*}{\Delta}.\Delta.\Delta.\Delta.\underset{*}{\Delta}.\underset{*}{\Delta}.\Delta.$ $\Delta.0.\underset{*}{\Delta}.\Delta.\Delta.0.\underset{*}{0}.\underset{*}{0}$	58	,,	210	·2	MS	+			extensive	,,	5·5 c.c.	present
42	B.T. (b)	$\Lambda.\Lambda.\Lambda.\underset{*}{0}.0.\Lambda.\Lambda.\underset{*}{\Lambda}.\Lambda.\Lambda.\underset{*}{\Lambda}.\Lambda.\underset{*}{\Lambda}.$ $\Lambda.\Lambda.\underset{*}{\Lambda}.\Lambda.\Lambda.\underset{*}{\Lambda}.\Lambda.\Lambda.\Lambda.\underset{*}{\Lambda}.\Lambda.\underset{*}{0}.\Lambda.$ $\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	62	,,	190 †310	·2 2·0	N N	N N	N N	14th day N N	- -	- -	- -	present
43	M.E. (a)	Δ.0.Δ.0.0.Δ.0.Δ.0.Δ.Δ.0.0. 0.0.0.0	32	,,	270	·2	LS	LS	+		extensive haemorrhagic	dark red	4·5 c.c.	0
44	S.E. (a)	$\Delta.\Delta.\underset{*}{0}.\underset{*}{0}.0.0$	22	,,	200	·2	MS	+			extensive	,,	·5 c.c.	present
Hospital														
45	F.H. (a)	Δ.Δ.0.0	18	,,	200	·2	SS	+			moderate	,,	-	0
46	C.B. (a)	Δ.0.0.0.0	15	,,	200	·2	MS	+			extensive	,,	-	0
47	M.S. (a)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.0.0.0	56	,,	220	·2	SS	LS	+		,,	,,	3·5 c.c.	0
48	R.C. (a)	$\Delta.0.\Delta.\underset{*}{\Delta}.\underset{*}{\Delta}.\Delta.0.0.\Delta.0.\underset{*}{0}$	41	,,	275	·2	MS	+			extensive haemorrhagic	red	4·8 c.c.	present

† Bacilli from 19th examination.

49	A.R. (a)	Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.0.0.0	51	acid	250	·2	N	N	N	14th day N	-	-	-	0
50	H.R. (a)	$\Delta.\Delta.\Delta.\underset{*}{0}.0.\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}.\Delta.0.\Delta.\Delta.$ $\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{0}.0$	99	,,	250	·2	SS	MS	+		extensive	dark red	4·5 c.c.	present
51	G.R. (b)	Δ.Δ.Δ.Δ.0.Δ.Δ.0.0.0.0	48	,,	250	·2	LS	LS	+		,,	,,	-	0
Sanatorium														
52	A.W. (a)	Δ.Δ	-	,,	250	·2	MS	+			extensive	,,	-	0
53	T.S. (b)	Δ.Δ.Δ.Δ.0	44	,,	200	·2	SS	MS	+		,,	,,	-	0
54	R.F. (a)	$\underset{*}{\Delta}.0.\underset{*}{0}.\underset{*}{0}$	33	,,	210	·2	MS	+			,,	,,	9·0 c.c.	present
55	D.W. (b)	Δ. Fatal	-	,,	250	·2	MS	LS	+		,,	,,	5·6 c.c.	-
56	E.H. (b)	Δ.0.0.0	12	,,	320	·2	MS	LS	+		,,	,,	3·4 c.c.	0
57	B.M. (b)	Δ.0.0.0	13	,,	320	·2	LS	+			extensive haemorrhagic	,,	-	0
58	H.H. (b)	Δ.Δ.0.0.0.0	12	,,	350	·2	LS	LS	+		extensive	,,	-	0
59	E.H. (b)	Δ.0.Δ.0.0.0	10	,,	330	·2	LS	LS	+		,,	,,	-	0
60	L.B. (a)	Δ.Δ.0.0.0	25	,,	320	·2	SS	+			slight	red	7·5 c.c.	0
St Matthew's School (Infants—Autumn)														
61	F.B. (a)	Δ. Fatal	-	,,	300	·3	SS	LS	LS	+	extensive	red	2·5 c.c.	-
62	K.R. (a)	Δ.0.0.Δ.0.0.0.0.0	30	,,	420	·3	SS	LS	+		,,	dark red	-	0
63	H.H. (a)	Δ.0.0.Δ.Δ.Δ.Δ.0.0.0.0	31	,,	430	·3	MS	LS	+		,,	red	9·7 c.c.	0
64	B.P. (a)	Δ.Δ.Δ.Δ.0.0.0	27	,,	250	·3	MS	+			,,	dark red	1·0 c.c.	0
65	L.K. (b)	$\underset{*}{\Delta}.\Delta.\underset{*}{\Delta}.0.0$	28	,,	450	·3	MS	LS	+		moderate	pink	6·2 c.c.	present
66	H.L. (b)	$\Delta.\Delta.0.\Delta.0.0.\underset{*}{0}$	28	,,	450	·3	SS	+			extensive	,,	1·2 c.c.	present
67	B.T. (b)	Δ.Δ.0.0.Δ.0.0.0	33	,,	400	·3	LS	+			,,	dark red	-	0
68	N.M. (b)	$\underset{*}{\Delta}.\Delta.0.\underset{*}{0}$	57	,,	350	·3	SS	+			,,	,,	2·2 c.c.	present
69	H.M. (b)	Δ.0.Δ	?	,,	246	·3	MS	+			,,	,,	-	0
70	G.P. (a)	Δ.Δ.Δ.Δ.0.0.0.0.0	18	,,	430	·3	MS	LS	+		,,	,,	-	0

No.	Initials	Results of consecutive examinations	Days during which diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations							Results of Autopsy			Pseudo-diphtheria bacillus
					Wt. of guinea-pig in grammes	Dose of 48 hours broth culture in c.c.	Results. Days				Oedema	Supra-renals	Fluid in pleura	
							I	II	III	IV				
Catherine Street School														
71	L.S. (a)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ. Δ.Δ.Δ.Δ.Δ.O.Δ.O.O.O.O	65	acid	250	·2	MS	+			extensive	red	-	0
72	D.G. (a)	$\Delta.\Delta.O.\Delta.\Delta.\Delta.\underset{*}{\Delta}.\underset{*}{\Delta}.\Delta.O.\underset{*}{O}.\underset{*}{\Delta}.\underset{*}{O}.$ $\underset{*}{O}.O$	33	,,	200	·3	SS	MS	+		,,	dark red	-	present
73	A.M. (a)	Δ.Δ.Δ.Δ.O.O.Δ.Δ.O.O.O	30	,,	250	·3	SS	+			moderate	,,	3·2 c.c.	0
74	E.B. (b)	Λ.O.O.O	13	,,	190	·3	N	N	N	14th day N	-	-	-	0
75	E.K. (b)	$\underset{*}{\Delta}.\Delta.\underset{*}{O}.O.\underset{*}{O}$	21	,,	245	·3	SS	+			extensive	dark red	3·2 c.c.	present
76	A.W. (b)	Δ.Δ.Δ.Δ.Δ.O.O.O.O.O.O	23	,,	200	·3	SS	+			moderate	,,	-	0
77	E.G. (b)	Δ.O.O	13	,,	160	·3	SS	+			extensive	,,	-	0
78	M.H. (b)	$\underset{*}{\Delta}.O.\Delta.\underset{*}{\Delta}.O.O.O.O$	23	,,	225	·3	SS	MS	+		extensive haemorrhagic	red	-	present
79	A.S. (b)	$\underset{*}{\Delta}.\underset{*}{\Delta}.\Delta.O.\Delta.O.O.O$	23	,,	205	·3	SS	LS	+		extensive	,,	-	present
80	M.S. (b)	$\underset{*}{\Delta}.\underset{*}{\Delta}.O.\Delta.\Delta.O.O.\Delta.O.O.O$	40	,,	160	·3	MS	+			moderate	dark red	-	present
81	N.S. (b)	$\underset{*}{\Delta}.\Delta.\underset{*}{O}.\Delta.\underset{*}{O}.O.O.O$	19	,,	200	·3	SS	+			extensive	,,	1·2 c.c.	present
82	N.B. (b)	Λ.O.O.O	13	,,	205	2·0	N	N	N	14th day N	-	-	-	0
83	M.A. (b)	$\Lambda.\Lambda.\Lambda.O.\underset{*}{O}.O$	16	,,	200	·3	N	N	N	14th day N	-	-	-	present
84	W.C. (b)	Δ.O.O	47	,,	250	·3	SS	+			extensive	dark red	-	0
85	G.T. (b)	Δ.O.O.O	8	,,	200	·3	SS	SS	SS	12th day +	slight	pink	5·8 c.c.	0
86	F.E. (b)	Δ.O.O.O	16	,,	230	·3	MS	+			extensive haemorrhagic	,,	-	0
87	L.G. (a)	$\underset{*}{O}.\Delta.\Delta.\Delta.\underset{*}{O}.\underset{*}{O}.O$	19	,,	200	·3	SS	+			,,	dark red	-	present
88	K.T. (a)	$\underset{*}{\Delta}.\underset{*}{O}.\underset{*}{\Delta}.\underset{*}{O}.\underset{*}{O}.\underset{*}{O}$	18	,,	150	·3	SS	MS	+		moderate	,,	-	present
89	G.T. (b)	Λ.O.O.O	20	,,	220	·3	SS	N	N	N	-	-	-	0
Abbey School														
90	M.A. (b)	Λ	?	,,	245	2·0	N	N	N	N	-	-	-	-
New Street School														
91	C.H. (b)	Λ.Λ.O.O.O	10	,,	200	2·0	N	N	N	N	-	-	-	0

St Barnabas School															
92	E.O.	(a)	Δ.Δ.Δ.Δ	81	acid	200	·2	MS	+			moderate	dark red	trace	0
93	D.O.	(a)	$\underset{*}{\Delta}.\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}$	57	,,	260	·2	SS	+			,,	,,	-	present
94	G.O.	(b)	$\underset{*}{\Delta}.\underset{*}{0}.\underset{*}{0}$	?	not tested										present
Post Office															
95	A.O.	(a)	Δ.Δ.Δ.Δ.Δ.Δ.0.0.Δ.0.0.0	16	acid	170	·3	MS	+			moderate	dark red	1·3 c.c.	0
96	J.H.	(a)	$\Lambda.\Lambda.0.\underset{*}{\Lambda}.\Lambda.\underset{*}{\Lambda}.\underset{*}{0}.\underset{*}{\Lambda}.\underset{*}{0}.0.\Lambda.0.0.0$	15	,,	300	·2	N	N	N	14th day N	-	-	-	present
						290	2·0	N	N	N	N	-	-	-	
97	F.H.	(b)	$\underset{*}{\Lambda}.\Lambda.\Lambda.\underset{*}{\Lambda}.\underset{*}{0}.0.\underset{*}{0}.0$	8	,,	260	2·0	SS	N	N	N	-	-	-	present
98	Y.B.	(b)	Λ.Λ.Λ.Λ.0.0.0	9	,,	300	2·0	N	N	N	N	-	-	-	0
99	R.L.	(b)	$\underset{*}{\Lambda}.0.\underset{*}{\Lambda}.\underset{*}{0}.\underset{*}{0}.0$	8	,,	200	2·0	N	N	N	N	-	-	-	present
Immediate contact unknown															
100	W.O.	(a)	$\Delta.\underset{*}{0}.\underset{*}{0}.0$	12	,,	210	·2	LS	+			extensive haemorrhagic	dark red	-	present
101	F.P.	(a)	Δ. Fatal	-	,,	220	·2	MS	MS	LS	+	extensive	red	6·3 c.c.	-
102	M.S.	(a)	Δ.Δ.0.0.Δ.Δ.Δ.0.0.0	35	,,	200	·2	LS	+			,,	dark red	1·5 c.c.	0
103	D.C.	(a)	Δ. Fatal	-	,,	205	·2	MS	LS	+		,,	,,	-	-
104	O.O.	(b)	Δ.Δ.Δ.Δ.Δ.0.0	70	,,	198	·3	MS	+			,,	,,	-	0
105	M.A.	(a)	Δ.Δ.0.0.Δ.0.Δ.0.0.0.0	58	,,	245	·3	SS	+			haemorrhagic	,,	1·2 c.c.	0
106	G.W.	(a)	Δ. Fatal	-	,,	245	·3	SS	+			extensive	,,	-	-
107	A.R.	(a)	$\Lambda.\Lambda.\underset{*}{\Lambda}.\Lambda.\underset{*}{0}.\underset{*}{0}.\underset{*}{0}$	11	,,	210	·2	N	N	N	14th day N	-	-	-	present
						200	2·0	N	N	N	N	-	-	-	
108	M.R.	(b)	Λ.Λ.0	20	,,	200	2·0	N	N	N	N	-	-	-	0
109	M.B.	(b)	Λ	-	,,	not tested									
110	W.R.	(b)	$\underset{*}{\Lambda}.\Lambda.\underset{*}{0}$	20	,,	205	2·0	N	N	N	N	-	-	-	present
111	D.R.	(a)	$0.\underset{*}{\Lambda}.\underset{*}{\Lambda}.\underset{*}{\Lambda}.\Lambda.\underset{*}{0}.\underset{*}{0}.\underset{*}{\Lambda}.\underset{*}{0}.\underset{*}{0}.0$	15	,,	220	2·0	N	N	N	N	-	-	-	present
112	E.Y.	(a)	0.0.Λ.0.Λ	?	,,	210	2·0	N	N	N	N	-	-	-	0
113	A.I.	(a)	Λ.Λ.0.0	8	,,	230	2·0	N	N	N	N	-	-	-	
114	N.E.	(a)	Λ.Λ.Λ.0	11	,,	300	2·0	N	N	N	N	-	-	-	0
115	J.C.	(a)	Λ.0.0.0	1	,,	200	2·0	N	N	N	N	-	-	-	0

Most satisfactory results have been obtained by isolating the infected persons until the disappearance of morphologically typical diphtheria bacilli from the throat and nose, or other situations in which they may happen to be found. When the bacilli have persisted for a long period and show no signs of becoming reduced in numbers, pure cultures should be obtained and animal inoculations made. Should the bacilli turn out to be devoid of virulence, and difficulty met with in enforcing isolation, the patient may be discharged with the knowledge that non-virulent diphtheria bacilli have seldom, if ever, been known to give rise to clinical diphtheria or any other lesion, when transferred from one individual to another.

Many observers have taken one negative culture to be sufficient evidence for the disappearance of the bacilli and for the remission of quarantine, but the need for at least two or three consecutive negative cultures, before the patient can with reasonable certainty be declared to be free from the bacilli, has been clearly established.

A negative culture followed by one or more positive cultures, in which diphtheria bacilli are found, is of fairly frequent occurrence in examinations for release from isolation. The preceding table shows the results of the examinations of 112 infected persons during an outbreak at Cambridge (1904). The nature, the result of each examination in regard to the presence of the diphtheria bacillus and Hofmann's bacillus, the period of persistence, and the effect of inoculation experiments are given in each case.

Premature misleading negative cultures may be due to several causes.

In the first place less care is probably exercised in taking the specimens than in procuring swabs for diagnosis when the physician is most anxious about the case. Then the membrane, which previously formed a guide to the proper point, has vanished, and the swab may not be taken from that part of the mouth in which the bacilli are present, and the bacilli are generally fewer in number. At the particular moment when the culture is taken they may have disappeared temporarily from the surface of that part of the mucous membrane reached by the swab, only those remaining which are ensconced in folds or follicles or other inaccessible recesses, from which they may emerge later.

Wolff (1895) was the first to call attention to the presence of diphtheria bacilli in accessory sinuses of the nose. He examined these sinuses in 22 fatal cases of diphtheria, and found diphtheria bacilli in 12 cases. They were discovered once in the frontal sinus, six times in seven examinations in the sphenoidal sinus, and twelve times out of fifteen examinations of the antrum.

Councilman, Mallory, and Pearce (1901) found diphtheria bacilli in 21 (40 %) out of 52 cases of inflammation of the antrum, and in 19 (51 %) out of 38 examples of middle ear disease following diphtheria. These observations though made on fatal cases point to the possibility of the bacilli lurking in such situations, and subsequently finding their way thence into the pharynx.

Even though the bacilli are present in the throat they may not be removed on the swab; or even if they reach the swab in small numbers they may fail to be transferred on to the serum. They may even reach the serum and either fail to grow, or be overgrown by other forms. Failure to grow may be due to the presence of an antiseptic, if the swab has been taken too soon after its application to the throat, or, if the medium has not been very recently prepared, to the surface being too dry, etc.

Overgrowth of the colonies of the diphtheria bacillus may be due to the presence of numerous other organisms, or more frequently to the presence of rapidly growing film-forming bacilli.

Finally, although present on the culture, the bacilli may not be detected owing to the smallness of their numbers or insufficient examination (see p. 334).

Hill (1898) observed that 40 % of those officially released by two consecutive negative cultures yielded single negative cultures followed by positive cultures during their course, but states that at the Boston City Hospital not more than 3 % of diphtheria cases yielded positive cultures following two consecutive negative cultures.

Cobbett's (1901) records show that in 37 % of cases negative were followed by positive cultures. Rickards (1906, p. 27) has stated that 22 %, out of nearly 2000 persons released by the laboratory, showed a negative culture followed by a positive culture. Many similar observations dealing with smaller numbers are to be found in the literature.

During the last four years the writer has made cultivations from the throats and noses of 331 persons, convalescents and infected contacts, until the diphtheria bacilli have disappeared as evidenced by three consecutive negative examinations. Of these persons 127 (38·3 %) gave premature negative followed by positive cultures, many of them on more than one occasion. A single negative followed by the finding of diphtheria bacilli occurred 112 times, two consecutive negatives 66 times, and three consecutive negatives 17 times. In one instance four consecutive negatives followed by a positive culture were recorded.

The opportunity but rarely occurs of examining after a period of several months persons who have been released from isolation after one or more negative bacteriological examinations. In a few instances the writer has been able to make such examinations and the results are given in the following table (p. 417).

The later cultures from 32 out of the 35 cases quoted showed no diphtheria bacilli. One of the children in whose throat the bacilli were found suffered from a second mild attack, for which no source of infection was traced. The other two were again discovered as healthy contacts in small outbreaks of the disease. In the first case diphtheria occurred amongst the members of the child's family, and in the second amongst the children attending the same school. In both cases it is probable that the child mentioned was the source of infection. The bacilli derived from both on the second occasion were fully virulent.

These records demonstrate that in about 40 % of cases one negative examination is misleading and that even two or three consecutive negatives cannot be entirely relied on as indicating the final disappearance of the diphtheria bacillus. Although it cannot, therefore, be claimed that danger is entirely removed by two, or even three consecutive negative examinations, it is certainly very much reduced.

However clearly the risk is realised of allowing convalescent patients and contacts infected with virulent diphtheria bacilli to mix with the normal population, the practical difficulties of enforcing prolonged isolation of any kind are often considerable. In the case of Infectious Diseases Hospitals, the wards of General Hospitals, Institutions and Schools, attended by the children of the well-to-do classes, little difficulty seems to have been encountered by most investigators.

The most serious opposition is likely to be encountered in dealing with isolated cases in adults and outbreaks amongst the scholars of the public elementary schools.

In the first case both the patient and the physician not unnaturally become impatient when the bacilli persist for an unusually long time. The former frequently fails to understand that he can still be a source of danger long after all symptoms of the disease have disappeared and when he appears to be in perfect health, and the latter chafes also under what he or his patients sometimes regard as an implied reflection on his skill, the delay in official release after he has declared the patient fully recovered appearing as a reversal of his decision. This idea is based on a misconception so obvious and simple that it would be unworthy of mention were it not so widespread. The procedure to be adopted in cases of this kind must of necessity depend on the circumstances in each

Number	Bacilli	Original period of persistence	Date of later examinations	Result of later examinations
Clinical Cases.				
1.	Virulent	18 days	5 months	Negative.
2.	,,	27 ,,	10 ,, 12 ,,	,, ,,
3.	,,	31 ,,	10 ,, 12 ,, 17 ,,	,, ,, ,,
4.	,,	18 ,,	16 ,,	,,
5.	,,	8 ,,	18 ,,	,,
6.	Not tested	21 ,,	19 ,,	,,
7.	Virulent	24 ,,	19 ,, 20 ,,	,, ,,
8.	,,	10 ,,	19 ,,	Bacilli present[1].
9.	,,	27 ,,	20 ,,	Negative.
10.	Not tested	17 ,,	21 ,,	,,
11.	Virulent	62 ,,	21 ,,	,,
Infected Contacts.				
12.	Virulent	25 ,,	2 ,,	,,
13.	Non-virulent	30 ,,	2 ,,	,,
14.	Virulent	47 ,,	4 ,, 5 ,, 23 ,,	Bacilli present[2]. ,, Negative.
15.	,,	40 ,,	5 ,,	,,
16.	,,	39 ,,	5 ,,	,,
17.	Non-virulent	9 ,,	6 ,,	,,
18.	,,	8 ,,	8 ,,	,,
19.	Virulent	9 ,,	7 ,, 18 ,, 19 ,, 37 ,,	Bacilli present[3]. Negative. ,, ,,
20.	Not tested	12 ,,	10 ,,	,,
21.	Virulent	33 ,,	10 ,, 11 ,, 17 ,,	,, ,, ,,
22.	,,	46 ,,	10 ,, 11 ,,	,, ,,
23.	,,	60 ,,	11 ,, 17 ,,	,, ,,
24.	,,	?	11 ,,	,,
25.	Non-virulent	8 ,,	14 ,,	,,
26.	Not tested	21 ,,	14 ,,	,,
27.	,,	30 ,,	19 ,, 20 ,,	,, ,,
28.	,,	9 ,,	19 ,, 25 ,,	,, ,,
29.	Non-virulent	122 ,,	19 ,,	,,
30.	,,	52 ,,	19 ,, 25 ,,	,, ,,
31.	Virulent	48 ,,	20 ,, 25 ,, 49 ,,	,, ,, ,,
32.	Non-virulent	?	20 ,,	,,
33.	Virulent	20 ,,	20 ,, 25 ,,	,, ,,
34.	,,	41 ,,	22 ,,	,,
35.	,,	24 ,,	30 ,,	,,

---

[1] Suffered from a second mild attack of diphtheria. No known cause of infection.

[2] Bacilli were still present when the patient was released. Four months after he developed the disease three cases occurred in his family.

[3] An outbreak occurred in the school attended by this child. Virulent bacilli, which lingered 33 days, were found in his throat. The disease broke out amongst his immediate neighbours, and he appears to have been the source of infection.

instance. If the patient's employment brings him into close contact with school children, or persons of susceptible age, it is clearly necessary to use every means to convince him of the necessity for keeping away from his work, until diphtheria bacilli can no longer be found. If on the other hand his employment is such that the probability of his spreading the disease is slight, it may be a mistaken policy to insist too much on his continued isolation, for by this action general objections may be aroused to the procedure in cases in which isolation is more necessary.

In the case of outbreaks of diphtheria amongst the scholars of the public elementary schools considerable difficulties may be encountered in efficiently isolating, and more especially in keeping isolated, the infected contacts and mild cases until free from infection, unless some plan of action has been arranged. In this case again compulsion may be fatal to success. Nothing can be done unless the people back up the measures. On the discovery of such cases the measures which have been arranged should be explained to the parents, and an attempt made to induce them *to allow the infected children to be satisfactorily isolated, until proved to be free from bacilli by three consecutive negative bacteriological examinations.* When diphtheria is prevalent the failure now and again to isolate and keep isolated a person in whom diphtheria bacilli have been found is not of great importance.

It is clearly impossible to bacteriologically examine everybody who may have by some chance caught the bacillus. And since some such persons must inevitably remain at large, one more or less will not greatly signify.

Nevertheless it is worth while taking a considerable amount of trouble, if we can only isolate a good proportion of these infectious persons, or at any rate keep them from school. That it is possible even in considerable outbreaks to carry into effect the measures which have been advocated, namely the examination of most persons who are likely to have come into contact with cases of diphtheria or infected persons, and isolate those who are discovered to be harbouring diphtheria bacilli, until evidence has been obtained by three consecutive negative bacteriological examinations of the disappearance of these bacilli, has been proved by the experience of the Colchester and Cambridge outbreaks (p. 427). In these outbreaks the hearty cooperation of the medical practitioners, school authorities, and the majority of parents was obtained, when the principles of the measures which were being undertaken had been explained to them. Wesbrook (1905) in Minnesota has also carried into effect similar measures in a number of instances.

This applies only to times when diphtheria is prevalent: at other times when none but sporadic cases occur it is possible no doubt to examine every contact, and very desirable to isolate all infected persons until free from diphtheria bacilli.

(6) *Records relating to the persistence of diphtheria bacilli in the throat and nose, etc.*

Some statistics have already been quoted (p. 236) showing that diphtheria bacilli may persist, and retain their full virulence, in the throats and noses of convalescents long after the disappearance of all clinical symptoms. Far longer periods of persistence have, however, been recorded by observers, who have only taken into consideration the morphological appearances of the bacilli. The following table shows the longest periods of persistence recorded by a number of workers.

Table showing the longest periods during which diphtheria bacilli have been found by various observers to persist in the secretions after an attack of diphtheria.

Observer	No. of days
Prip (1901)	669
Meyer (1898)	547
Le Gendre & Pochon (1895)	458
Fibiger (1897)	276
Schäfer (1895)	230
Woodhead (1901)	210
Belfanti (1894)	210
Dowson (1893)	200
Massachusetts (1900)	185
Macgregor (1898)	183
Park (1900)	183
Williams (1896)	157
Jensen (1897)	183
Hewlett (1896)	154
Russell (1899)	137
Graham-Smith (1904)	122
Wesbrook (1905)	109
Cobbett (1901)	108
Minnesota (1900)	97
Müller (1897)	75
Abel (1894)	65
Washbourn & Hopwood (1895)	63
Martha (1895)	63
Glucksmann (1897)	49
Sevestre & Mery (1895)	49
Symes (1895)	49
Gladin (1895)	45

Although exceptional cases such as these come under the notice of nearly all observers, diphtheria bacilli as a rule can no longer be found after a few weeks, and in many cases even a few days after the disappearance of the local lesions. The mean period of their persistence varies to some extent according to the observations of different workers.

Woodhead (1901) during 1895–6 examined a large number of cases treated at the Metropolitan Asylums' Board Hospitals, and found that the mean period of persistence was 51 days. In his tables he differentiates

Table showing the persistence of morphologically typical diphtheria bacilli in the secretions[1].

Period in days of persistence of bacilli	Woodhead	Massachusetts Board of Health (1896—1905)	Wesbrook (1900)	Park	Prip (1901)	Minnesota Board of Health	Glucksmann	Scheller (1905)	Tobieson	Total
1—5	—	66	—	304	—	—	3	—	10	1073 (11·8)
5—10	229	65	—	176	118	17	—	75	10	
11—15	—	400	17	100	—	23	26	264	1	1846 (19·8)
16—20	353	518	27	—	106	23	—	—	1	
21—25	—	271	—	12	—	57	29	119	—	1825 (19·8)
26—30	710	490	22	4	51	48	11	—	—	
31—35	—	304	10	—	—	—	9	62	1	1494 (16·3)
36—40	947	140	—	—	20	21	—	—	—	
41—45	—	47	—	—	—	—	4	35	—	1023 (11·2)
46—50	851	78	—	—	—	6	2	—	—	
51—55	—	8	—	—	—	—	—	26	—	653 (7·2)
56—60	526	39	—	2	41	11	—	—	—	
61—70	375	35	—	—	11	—	—	18	—	428 (4·7)
71—80	249	17	—	—	—	—	—	—	—	266 (2·9)
81—90	170	11	—	—	4	—	—	—	—	185 (2·0)
91—100	107	8	—	—	—	—	—	8	—	125 (1·4)
101—110	81	4	—	—	6	—	—	—	—	85 (·9)
111—120	69	1	—	—	2	—	—	—	—	72 (·8)
121—130	30	—	—	—	5	—	—	—	—	30 (·3)
131—140	16	2	—	—	—	—	—	—	—	18 (·2)
141—150	11	1	—	—	—	—	—	—	—	12 (·13)
151—160	5	—	—	—	2	—	—	—	—	5 (·05)
161—170	7	—	—	—	—	—	—	—	—	7 (·07)
171—180	2	—	—	—	—	—	—	—	—	2 (·02)
181—190	2	1	—	—	—	—	—	—	—	3 (·03)
191—200	2	—	—	—	—	—	—	—	—	2 (·02)
201—210	2	—	—	—	—	—	—	—	—	2 (·02)
Over 210					3[3]					
	4744	2506	87[2]	598	309	208	84	607	24	9080
Mean	51·2	28·0	29	6·6	24·7	26·8	24·8	20·9	8·4	38·4

between patients treated with and without antitoxin, but no appreciable difference was observed between them. No other recorded observations show such a long mean period of persistence, the longest being 29 days and the shortest 6·6 days.

The figures given by several authorities, most of whom regarded one negative examination as sufficient evidence of the disappearance of the bacilli, are summarised in the preceding table, p. 420. These records very conclusively demonstrate the impossibility of choosing any time limit by which a patient might with any reasonable certainty be considered free from infection.

---

[1] Some of these observers took one negative examination as sufficient evidence for the disappearance of the bacilli and some two consecutive negative examinations.

[2] In 12 cases the bacilli disappeared after the 35th day of the disease.

[3] Over 8, 11, and 22 months. Two sets of observations by Prip are included. In one set the figures are given in months.

The records which are quoted above refer only to the microscopical examination of cultures taken from convalescent patients. They show that in three weeks about 30 % of patients are free from morphologically typical diphtheria bacilli. In 20 % the bacilli persist for four weeks, in 16 % for five weeks, and in 11 % for seven weeks. One per cent. harbour them for 15 weeks, and in exceptional cases they remain in the throat for thirty weeks, though even more prolonged periods of persistence are recorded.

It is of considerable interest to inquire whether any difference exists between the periods of persistence of virulent and non-virulent bacilli in clinical cases and contacts respectively. On these points the number of observations is very limited, and no marked differences are to be seen. The following table has been constructed from Cobbett's (1901) published observations and the writer's (published and unpublished), and includes only the records relating to cases in which three consecutive negative cultures were eventually obtained, and in which the bacilli were tested for virulence.

Period of persistence of bacilli in days	Notified cases		Infected healthy contacts	
	Bacilli virulent	Bacilli non-virulent	Bacilli virulent	Bacilli non-virulent
1—5	2	2	—	1
6—10	6	1	7	7
11—15	5	4	6	17
16—20	8	—	3	5
21—25	10	1	6	—
26—30	12	2	5	2
31—35	6	—	2	3
36—40	2	—	2	2
41—45	2	—	4	1
46—50	2	—	4	1
51—55	1	1	1	3
56—60	4	—	3	1
61—70	3	—	3	1
71—80	1	—	1	—
81—90	2	—	3	—
91—100	1	—	—	—
101—110	—	—	—	2
	67	11	50	46
Mean period	31·6 days	18·5	36·4	30·0

Certain persons were notified on very slight clinical grounds because diphtheria bacilli were found on bacteriological examination, but none of them suffered from typical diphtheria. The majority of the figures in the second column refer to such cases.

Persons harbouring virulent and non-virulent bacilli and clinical cases and contacts are separately considered.

According to the observations of Meikle (1906) the duration of the persistence of the bacilli is not appreciably affected by season, sex, age, number of days ill before admission, the amount and position of the membrane, the amount of antitoxin given, or the date of disappearance of the membrane.

The various statistics which have been quoted prove that diphtheria bacilli may persist for very long periods in the throats and noses of both convalescent patients and infected contacts, and retain their full virulence. They also show that in any given case it is impossible to foretell how long they will persist. Isolation for fixed periods of time can therefore be of little value, and gives rise to a false sense of security.

(7) *Attempts to hasten the disappearance of the bacilli.*

Since the discovery was first made that diphtheria bacilli remain in the throat after the clinical lesions have disappeared, efforts have been directed towards hastening their departure by the application of antiseptics. Great numbers of prescriptions have been recommended for use in various forms, as sprays, gargles, inhalations and as direct applications to the lesions. All these various methods and formulae have their advocates who consider that by their means the duration of the persistence of the bacilli is shortened, at least in many instances. During the last four years many of the methods which have been recommended have been given prolonged trials, but none have been found which give uniformly satisfactory results. In some instances good results seemed to follow, in others this was certainly not the case, and the writer has come to the conclusion that by antiseptic treatment the duration of the stay of the bacilli is not materially affected. Meikle (1906, p. 524) has recently made extensive observations on this subject, and gives the results in tabular form. He states that "no one antiseptic seems to be much better than another." Several other observers have also arrived at the same conclusion.

Some form of antiseptic treatment is however desirable for several reasons. In the first place the duration may perhaps be shortened in some cases, and in the second the patients and their friends are better satisfied if some attempt is apparently being made to shorten the period of isolation. Thirdly, the application of antiseptics probably hinders the multiplication of the bacilli on the mucous surfaces, and also diminishes their vitality outside the body. Consequently the treatment, although it is unable to affect the bacilli, which are present in the crypts of the tonsils and other out-of-the-way situations, and therefore does not reduce the period of infectivity of the patient, is of benefit in limiting the power of infecting others, by its action on the superficial bacilli.

When swabs are taken from patients undergoing such treatment an interval of several hours ought to elapse between the application of the antiseptic and the taking of the swab, in order to allow the bacilli which are lurking in the crypts, etc., to regain the surface, and to prevent any recently applied antiseptic producing an inhibitory action on the culture.

It has occasionally been noticed that after an inflammatory lesion due to some other agent than the diphtheria bacillus these organisms disappear from the throats of healthy infected persons. In the case of a man, in whom the bacilli persisted in a few large crypts for an unusually long time after an attack of diphtheria, an attempt was made to hasten their departure by the application of irritants to these crypts. By means of small bent swabs small quantities of mustard oil were applied on several occasions to each of the enlarged crypts. This procedure resulted in an acute inflammatory process in the superficial portions of the crypts, but the bacilli nevertheless persisted in the deeper portions.

Wassermann (1902) has tried the experiment of giving tabloids made from the serum of horses immunised against several strains of living diphtheria bacilli. He claims to have been successful in hastening the disappearance of the bacilli by this method.

L. Martin (1903), by injecting the bacillary bodies intravenously or intraperitoneally into horses, obtained sera with marked agglutinating properties. He claims that this serum has, when applied locally, the property of causing a rapid decrease in the number of living bacilli in the throat. The best results are obtained by incorporating the dried serum with gum, and using the remedy in the form of pastilles.

In many instances the removal of enlarged tonsils, adenoids or nasal growths, which provide the bacilli with situations where they may multiply unharmed by antiseptic applications, might be of great benefit in hastening their disappearance. Pegler (1905) stated that "he had several times found that the cause of this lingering presence of bacilli could be removed by carefully extirpating by means of morcellement every trace of unhealthy tonsil tissue in which crypts could be discovered." In other cases he had found adenoid, or naso-pharyngeal tonsillar tissue to be the offending cause of the trouble.

(8) *The results of the isolation of convalescents, mild cases, and infected contacts until the disappearance of diphtheria bacilli.*

(*a*) *Families.*

Park (1894) investigated the throats of the members of 14 infected families in which there were 48 children. In 50 % of these virulent diphtheria bacilli were found; 40 % developed later, to a greater or less extent, the lesions of diphtheria. It is noted in Park's paper that, in these families, the conditions were the best possible for the transmission of the bacilli from one to the other. In families where the case of diphtheria was well isolated, the bacilli were found in less than 10 % of the children.

(*b*) *Schools.*

One of the first attempts to stamp out diphtheria by the isolation of infected contacts was brilliantly successful. The credit is due to Fibiger (1897), who dealt with an outbreak in a gymnasium at Herlrifsholm in the Island of Zealand. In December 1894 there were three cases of diphtheria. Then came the Christmas holidays, during which the rooms, bed clothes, etc., of the patients were twice disinfected before the school reassembled. The scholars returned on January 7th, and on the 13th, a case of diphtheria occurred, quickly followed by five others. A bacteriological examination of all the healthy persons (134) was undertaken, with the result that diphtheria-like bacilli were found in 22. Further investigations were made to differentiate between the true and pseudo-diphtheria bacilli. It was then found that those (10) who harboured the true diphtheria bacilli were children who shared the same rooms as the diphtheria patients, and as a rule were the next neighbours. One of these children had suffered from the disease in December, but had recovered. One case occurred in a class which had not been affected, and it turned out that the brother who belonged to another class had carried the infection.

After the isolation of the diphtheria bacilli-carrying individuals no cases occurred for 1½ years. Some of these infected contacts retained the bacilli for long periods; in one case virulent bacilli being found for nine months.

Gabritschewsky (1901) on two occasions checked outbreaks of diphtheria in large schools by the examination of the scholars, and the isolation of all in whom diphtheria bacilli were discovered.

Burnett's (1900) case, which has already been quoted (p. 311), affords an excellent example of diphtheria reintroduced into a school by a convalescent patient. In this case the patient was thought to be free of diphtheria bacilli, but they were again found after his return to school. Peck (1901) also gives an instance illustrating the reintroduction of the disease by a contact. In a school with 50 boarders and 50 day scholars two boarders were found to have sore throats on October 5th. Swabs were taken and diphtheria bacilli discovered. The day scholars were immediately dispersed, and one boarder (A. B.) was allowed to go home on the understanding that she was not to return till bacteriologically free. A third case occurred on October 8th. All the persons in the house, including the boarders, teachers, and servants were then examined, and all but two were found to be free from diphtheria bacilli. The three cases and two infected contacts were isolated.

On November 5th all the scholars reassembled and all were bacteriologically free. A. B. returned on November 12th but went home on November 16th, and on November 19th developed diphtheria. It was then ascertained that she had not been examined, but had been using an antiseptic spray. On November 18th five boarders were suffering from slight ailments and three were found to have diphtheria bacilli; swabs from the rest of the school showed 28 children harbouring the bacilli.

(c) *Institutions.*

Berry (1900) gives a very interesting account of an outbreak of diphtheria at the London Orphan Asylum in 1898, which was arrested by the examination of the children and the isolation of those infected. At that time there were 350 boys and 200 girls in the Asylum, who were kept apart, and except at the Summer and Christmas holidays had little communication with the outside world. A case of true diphtheria occurred amongst the girls shortly after the Christmas holidays (Feb. 25th). A series of mild sore throats followed till

March 27th, when another case of clinical diphtheria occurred. At the same time three children with sore throats were found to be harbouring diphtheria bacilli, and were isolated. Nevertheless numerous cases of sore throat followed until April 30th, when five children developed diphtheria. At this time there were 76 cases of sore throat. Washbourn then examined all children (142) showing any abnormality of the throat, and discovered diphtheria bacilli in 19 of them. These children were isolated and not allowed to mix with the healthy girls until free from diphtheria bacilli. From that time no further cases of diphtheria or sore throat occurred.

Wesbrook (1905) records the successful treatment of an outbreak amongst the inmates of the State School for the Deaf at Faribault in which unusual difficulties had to be overcome. Twenty clinical cases were under treatment amongst the 280 inmates when the measures were put into force. All persons connected with the institution were examined and all infected individuals, who included 28 % of the children, isolated. All who showed suspicious bacilli (other types than D, C, or A) were re-examined, and if they failed to show D, C, or A on the second examination were released.

The infected individuals were not allowed to mix with the others until three successive negative throat and nose cultures had been recorded. Only two new clinical cases occurred after the measures were put into force and the disease was effectually eliminated, although there were many difficulties incidental to overcrowding, some difficulties in instructing the pupils, and some due to the facts that the pathological conditions of the nose and throat which originally gave rise to the deafness were favourable to diphtheria infection, and that the use of the sign language employed increased opportunities for infection from the hands.

(*d*) *Hospital wards.* (See also Chapter XII.)

Garratt and Washbourn (1899) from their observations at the London Fever Hospital from March 1896 to December 1898 come to the conclusion "that post-scarlatinal diphtheria is due to the introduction of unrecognised cases of diphtheria into scarlet fever wards, and that this can only be obviated by systematic bacteriological examinations of all cases on admission and by the separation of those in whose throats diphtheria bacilli are found." They considered that Hofmann's bacillus had no connection with diphtheria, and did not separate persons showing this organism.

These observers succeeded in producing a great reduction of post-scarlatinal diphtheria by isolating those cases in which diphtheria bacilli were found (see p. 439).

Soerensen (1898) had in the previous year called attention to the rapidity with which diphtheria bacilli spread amongst scarlet fever patients, when a case of diphtheria or persons infected with diphtheria bacilli are introduced into a ward.

Hutchens (1906) records an outbreak of diphtheria in a scarlet-fever ward. On examining the throats of all the inmates diphtheria bacilli were found in 23 % and Hofmann's bacilli alone in about one-half the remainder. "The former were all isolated, and no further cases occurred."

The instance given by Cuno (1902) of diphtheria spread in wards by a sister harbouring virulent diphtheria bacilli has already been quoted (p. 316). On the discovery of this fact and the isolation of the sister the outbreak came to an end.

Peters (5. VIII. 1907) completely checked an outbreak amongst the members of the staff of the Nottingham General Hospital by the isolation of infected contacts.

(e) *Towns.*

Two remarkable instances of the effects of efficient examination and quarantine in dealing with considerable outbreaks are given in the Report of the Massachusetts State Board of Health (1900). At Waltham on the opening of the schools an epidemic began which spread to all sections of the city. In November there were at one time 50 cases, and several new cases were being reported daily. The throat of each school child in the city was examined and cultures taken from all suspicious cases. By this means 22 children were found to be suffering from mild diphtheria. "These children were isolated and kept in quarantine until cultures showed them to be free from infection. The effect of this procedure upon the spread of the epidemic was soon apparent, and before the Christmas vacation the number of new cases was comparatively small."

At West Springfield during the last five months of 1899, 29, 44, 37, 25 and 24 cases had been notified, and 18 cases occurred in January 1900. Quarantine, until cultures showed the absence of diphtheria bacilli, was advised by the State Board of Health at the beginning of February. Only two cases occurred in February and none in March.

Cobbett (1901) in 1900 dealt with a serious outbreak of diphtheria at Cambridge by the examination of contacts and the isolation at an Isolation Home of almost all those found to be infected. Prophylactic injections of antitoxin were also given to a considerable [illegible] of the latter. The outbreak soon subsided after these measures had been put into operation. He emphasises the fact that diphtheria bacilli were only found in actual cases of diphtheria, or among those who had come directly into contact with such cases. A recurrence of the disease in the spring of the following year was also similarly treated.

Cobbett (23 XI. 1901) considers "that the duty of discovering, isolating and disinfecting the former class of persons (infected contacts) is becoming more and more the urgent duty of the sanitary authority. For the fact that they are not scattered broadcast throughout the community as was once supposed, but are confined to the class of persons conveniently called 'contacts,' renders their discovery a practical possibility and offers a fair prospect that at least the great majority of them may in the near future be subjected to isolation and antiseptic treatment with immense advantage to the public health."

Exactly similar measures were employed in combating a widespread outbreak of diphtheria at Colchester in 1901 by Graham-Smith (1902), but in this instance a very large number of cases had occurred before the measures were put into force.

The accompanying chart (p. 429) illustrates better than any description the success of the system. As may be seen in spite of the closure of the schools on June 15th, six cases a week on the average continued to be notified for 18 weeks up to October 19th. By this date all the probable contacts had been examined and the infected children quarantined. The result was that, in spite of the re-opening of the schools at a time of year when diphtheria might be expected to increase, the disease almost completely disappeared. A small outbreak occurred at the Kendall Road School, which was immediately stamped out by the re-examination of this school. Three other cases of true diphtheria also were notified during the remaining 10 weeks of the year.

In 1903 Graham-Smith (1904) again employed the same measures in an outbreak at Cambridge. In this case the disease occurred amongst the scholars of certain schools (3) and amongst the inmates of certain institutions (3). Each of these outbreaks was successfully dealt with in turn[1].

Wesbrook (1905) reports several widespread epidemics most successfully combated by following up and examining all cases, and all rumours and suspicions of possible cases. He also examined all school children, and excluded from school each child and each teacher who showed

---

[1] See Tables, pp. 408—413.

Chart showing the results of the isolation of mild unrecognised cases and infected contacts amongst the School Children of Colchester during an outbreak in 1901.

Schools	Jan. 5	,, 12	,, 19	,, 26	Feb. 2	,, 9	,, 16	,, 23	March 2	,, 9	,, 16	,, 23	,, 30
St John's Green					●								
Barrack Street													
North Street													
Kendall Road						●						●	
Central													
Camp						●						●	
Old Heath	●												
Magdalen Street													
St Paul's													
Wesleyan													
Private													
St Mary's													
Other Schools													

Schools	April 6	,, 13	,, 20	,, 27	May 4	,, 11	,, 18	,, 25	June 1	,, 8	,, 15	Schools closed ,, 22	,, 29
St John's Green				●	●			●●●●	●●	●	●●●●●●		●●●
Barrack Street						●	●●●	●●	●	●	●●	●●●	
North Street													●●
Kendall Road						●●	●	●●●			●●●●●●●●●●●●	●●●	●●
Central											●		●
Camp													
Old Heath													
Magdalen Street											●		●
St Paul's	●												
Wesleyan													
Private													●
St Mary's													
Other Schools													

Schools	July 6	,, 13	,, 20	,, 27	Aug. 3	,, 10	,, 17	,, 24	,, 31	Sept. 7	,, 14	Half the schools opened ,, 21	,, 28
St John's Green	●●●	●●●	●	●		●	●		●				●
Barrack Street	●		●●●	●	●●●	●	●		●	●●●	●●●	●	●●
North Street		●			●		●	●●●●●●					
Kendall Road		●	●	●	●			●					
Central	●	●			●		●						
Camp													●
Old Heath			●										
Magdalen Street				●●								●●	
St Paul's													
Wesleyan	●			●							●		
Private				●						●	●		
St Mary's	●	●											
Other Schools				●								●●	●

Schools	All schools opened Oct. 5	,, 12	,, 19	Examination of scholars completed ,, 26	Nov. 2	,, 9	,, 16	,, 23	,, 30	Dec. 7	,, 14	,, 21	,, 28
St John's Green	×		●●										
Barrack Street	●●●	●●●●		×							●×	×	
North Street	●●		●										
Kendall Road				×		●×	●●●	●	●✱				
Central	●●												
Camp													
Old Heath													
Magdalen Street													
St Paul's													
Wesleyan													
Private		●●	●										
St Mary's			●				●●						
Other Schools													

● indicates a notified case of diphtheria.

× indicates a notified case of diphtheria in which no diphtheria bacilli were found.

✱ re-examination of Kendall Road School completed.

diphtheria bacilli, until he and all his family were found free from infection. In 1900 Park Rapids, a town of almost 1000 inhabitants, was badly infected, not only school children, but adults being attacked. As a result of the measures taken "diphtheria disappeared under the conditions and at the time of year when there was every reason to expect its increase. The willing co-operation of the Town Council, School Board and general public was easily secured and retained."

"Similar methods have been employed and comparable results obtained in a number of other places, notably Grand Rapids, Benson, Bowerville, Duluth, Robbinsdale, Heron Lake, etc." He concludes by observing that "in Minnesota public sentiment supports such methods and at times demands them."

The sudden cessation of considerable outbreaks without the application of such measures has frequently been noticed, and the fact has occasionally been used as an argument against the necessity for their application.

The Prophylactic Injection of Antitoxin.

Several investigators have claimed excellent results from the prophylactic injection of antitoxin into the healthy members of infected households and into "infected contacts." There can be little doubt of the value of this treatment of persons exhibiting symptoms, which may be due to commencing diphtheria, in whose throats and noses diphtheria bacilli have been demonstrated. Except under certain conditions the injection of healthy infected contacts can scarcely be recommended in considerable outbreaks. Although a certain number of these persons do develop clinical diphtheria some days after the bacilli have been discovered in their throats, the majority show no symptoms of the disease. The attempt to inject all the infected contacts adds greatly to the labours of the workers, and is always liable to arouse opposition, not only to prophylactic injections, but to the measures in general amongst ignorant or prejudiced parents, especially if the children suffer any temporary inconvenience. The expense is also necessarily heavy and falls on the sanitary authority. A further and more serious objection to the practice is that it leads to a feeling of false security. The injected individuals do not often contract diphtheria, but nevertheless retain the bacilli in their throats as long as those who have not been injected, and unless carefully isolated are liable to act as carriers of the disease. Instances are also recorded (Jump, 1902, etc.) of persons who have received prophylactic injections developing the disease weeks after infection without reinfection. Peters (1907) found that seven out of 21 infected contacts, who received prophylactic doses of antitoxin (500 units), developed diphtheria within three weeks. If all the infected contacts that are known are under supervision, and instructions have been given to the parents or guardians immediately to notify any ailments amongst them to the medical authorities, prophylactic injections are unnecessary, since antitoxin can be at once given on the occurrence of suspicious symptoms. On the other hand prophylactic injections of antitoxin ought to be given in cases where medical attendance cannot easily be procured and the parents are likely to await the development of a serious condition before sending for the medical man.

Summary of Chapter XI.

The same general preventive measures are employed in dealing with outbreaks of diphtheria as in combating other infectious diseases. The measures include the notification of all persons suffering from typical and atypical forms of the disease, and their efficient isolation, and the disinfection of all articles used by them. Most authorities consider that healthy persons, found to be harbouring diphtheria bacilli, should not be notified but treated separately. During outbreaks the public elementary schools are generally closed, but this ought to be preceded by the bacteriological examination of the children. Special preventive measures based on bacteriological findings are now frequently employed, especially in the case of schools, hospital wards and institutions, and have also been successfully used in dealing with larger outbreaks in towns. These measures include the bacteriological examination of all suspected cases, and of those who have come into contact with them, and the isolation, or at least exclusion from intercourse with the susceptible members of the community, of all found to be harbouring diphtheria bacilli. Isolation should be enforced, whenever possible, until the bacilli have disappeared as evidenced by at least two, and if possible three, consecutive negative bacteriological examinations. Occasionally the bacilli persist for very long periods of time, particularly in persons with large tonsils, etc. None of the numerous antiseptic applications, which have been employed, have proved very successful in hastening their disappearance. Except when combined with efficient bacteriological examinations indiscriminate prophylactic injections of antitoxin cannot be recommended.

CHAPTER XII.

POST-SCARLATINAL DIPHTHERIA.

The prevalence of post-scarlatinal diphtheria. Origin. Treatment in the same hospitals of scarlet fever and diphtheria. Introduction of unrecognised cases. Results of bacteriological examinations. Spread of diphtheria in scarlet-fever wards. Prevention.

The Prevalence of Post-scarlatinal Diphtheria.

THE liability of scarlet fever patients to develop diphtheria has long been known, and the literature relating to the connection between the two diseases is very extensive. Although patients suffering from diphtheria may subsequently develop scarlet fever, or both diseases appear simultaneously in one subject, in the great majority of instances in which these two diseases occur in the same patient diphtheria follows the attack of scarlet fever.

Numerous recent English and foreign statistics show that scarlet fever patients treated in hospitals are liable to develop diphtheria in greater or less numbers, and their liability to this complication is universally recognised. Consequently it seems unnecessary to give at length the figures relating to this subject.

For several years returns have been made by the hospitals of the Metropolitan Asylums Board on the subject of this complication. Previous to 1895 only cases of scarlet fever which showed clinical diphtheria, having membrane on the fauces or exhibiting laryngeal symptoms, were designated post-scarlatinal diphtheria; since that year all cases of secondary throat illness associated with the diphtheria bacillus have been returned as diphtherial, including those which would from the clinical appearance alone have been regarded as simple tonsilitis.

In the year 1895 there consequently appears a sudden and large increase in the incidence of secondary diphtheria among the patients in the scarlet fever wards of these hospitals.

	1891	1892	1893	1894	1895	1896	1897	1898	1899	1900
Scarlet fever cases completed	5,444	11,326	14,867	12,637	10,422	15,054	15,250	12,771	13,327	10,749
Cases of post-scarlatinal diphtheria	99	217	207	210	453	705	796	661	692	405
Percentage incidence	1·8	1·9	1·3	1·6	4·3	4·6	5·2	5·1	5·1	3·7

Pugh (1902) worked out the seasonal incidence of these cases for the five years 1896–1900. He states that "if the number of cases of post-scarlatinal diphtheria, developing in each month in the five years under consideration, be averaged and corrected for the mean daily number of scarlet fever patients under treatment in each of these months, it will be found that the incidence of this complication does not follow the seasonal variation of diphtheria in the Metropolis, nor does it appear to depend on whether the hospitals are full or the reverse."

"In calculating the incidence of post-scarlatinal diphtheria on the the number of patients discharged and dead an important correction is necessary. Returns made in the years 1899 and 1900 show that the patients treated to recovery or death in the Board's town institutions (acute hospitals) have been in hospital about 68 days on the average, while patients who have completed their recovery or died at the convalescent hospitals have been, on the average, 31 days at the town hospital and about 48 days at the convalescent institution. If the calculation be made on 'patient days' (on the 'foot-pound' principle), it will be found that the liability to post-scarlatinal diphtheria at the convalescent hospitals is about two and one-third times as great as at the town hospitals."

The origin of post-scarlatinal diphtheria.

Pugh (1902) recently discussed the origin and mode of dissemination of post-scarlatinal diphtheria in the Metropolitan Asylums Board Hospitals, basing his conclusions on own observations, the statistics of the Board, and the previous literature. This section, in which the principal reasons are considered which have been suggested for the occurrence of secondary diphtheria in scarlet fever wards, is mainly based on this author's paper.

(1) *Sanitary defects.*

"As advances have been made in our knowledge of the bacterial origin of diphtheria, the belief, once generally held, that defective drainage played an important part in disseminating this disease has gradually waned. However, it may be as well to recall that Sweeting in 1893, investigated this point in connection with the Board's hospitals, and found that post-scarlatinal diphtheria had prevailed in like degree in hospitals with ventilated and in those with unventilated soil-pipes; in hospitals with automatic flushing apparatus, and in hospitals without such appliances; in hospitals with elaborate systems of ventilation and disconnection and in hospitals where these were of the most meagre and incomplete kind. In fact, the diversity was so great that no common factor of drainage defect could be pointed to as explaining the long continued yearly recurrence of this condition of post-scarlatinal diphtheria."

(2) *The treatment in the same hospital of the two diseases.*

"It is but natural that a layman, unacquainted with the administration of a fever hospital, should, when he hears that his child, convalescent from scarlet fever, has developed diphtheria, forthwith concludes that infection has been derived from cases of diphtheria treated in the same hospital." This opinion has to some extent been shared by members of the medical profession.

Now if, as supporters of this theory have held, the treating in the same hospital of the two diseases is the main cause of post-scarlatinal diphtheria, one would expect it to be of comparatively rare occurrence in hospitals reserved entirely for the treatment of scarlet fever. That this is not so is evident from the experience of the North Eastern and Gore Farm Hospitals. The former during the five years 1896–1900 received only patients certified to be suffering from scarlet fever, but 160 cases of secondary diphtheria occurred, while at the latter, which during 1897–8 received scarlet fever convalescents only, 273 cases of diphtheria were recorded.

This point is further emphasised by a comparison of the incidence of post-scarlatinal diphtheria at the Northern Hospital and Gore Farm during the years 1896–8. At the former convalescents from both diseases were admitted and at the latter only scarlet fever convalescents, yet the incidence of post-scarlatinal diphtheria in the two institutions was almost identical, namely 4·9 % at the former and 4·5 % at the latter.

Pugh (1902, p. 292) remarks that "it may, therefore, be regarded as proved, so far as statistics are able to help one, that the aggregation upon the same site of the two diseases is not an important factor in the etiology of post-scarlatinal diphtheria. Indeed, since diphtheria spreads solely through intimate contact with the source of infection, it can extend to the scarlet fever wards only in consequence of imperfect separation of the convalescents or through conveyance there by members of the staff."

The first of these two means of infection, if it occurs, can easily be remedied, and owing to the methods usually adopted can scarcely be of much importance. Practically the only persons involved are therefore the medical officers and the nurses. The former, however, are not brought into sufficiently close contact with their patients to encourage the belief that they serve as sources of infection with any degree of frequency. The nurses, on the other hand, owing to the more intimate relations which exist between them and the children, are more important factors in the spread of the disease.

Many instances are recorded in the literature on diphtheria of the infection of doctors and nurses with diphtheria bacilli, and instances have already been quoted (p. 316) of the spread of the disease by this means.

Pugh (p. 292) cites an interesting example: "Prior to the opening of our diphtheria wards there was an isolation building in this hospital, used for cases erroneously diagnosed as scarlet fever, containing four separate rooms, which were looked after by a single nurse. In one was a child with bronchitis; in another a patient suffering from diphtheria. The latter died on November 18th, five days after admission. On December 3rd, the bronchitic child, who had not yet left his bed, developed laryngeal diphtheria necessitating tracheotomy. No source of infection appeared possible save by the medical or nursing staff. Cultures were made from the throats of all who had been in contact with the child, and from one nurse, who had been in attendance on the diphtheria case of a fortnight before, virulent diphtheria bacilli were obtained. She had throughout had no sore throat, and the tonsils showed only chronic enlargement."

(3) *The introduction of unrecognised diphtheria.*

According to most authorities the introduction of mild and unrecognised cases of diphtheria into scarlet fever wards is the cause of post-scarlatinal diphtheria. "Among the cases received into fever hospitals certified as scarlet fever, a few can be readily recognised clinically as uncomplicated diphtheria. A larger class is that in which there is on admission evidence only of tonsilitis, for patients have not infrequently lost by the time they arrive at the hospital the other signs upon which the practitioner founded his diagnosis, and yet many of these are proved subsequently, by the occurrence of desquamation, to be suffering from the disease certified. Owing to the limited number of isolation rooms, a considerable proportion of these cases of apparent tonsilitis are admitted for observation into the scarlet fever wards, and one of mild diphtheria might thus be the origin of an outbreak of post-scarlatinal diphtheria.

Another class of case is that of double infection. Occasionally in addition to the signs of scarlet fever, the patient presents undoubted diphtheritic membrane in the throat. These cases, however, must not be confounded with a much larger number in whom a condition of throat more or less simulating diphtheria is found....Cases of the combined diseases may occasionally be admitted in which the local evidence of diphtheria is so slight, or else so masked by the lesions of scarlet fever, as to escape recognition. Finally, as is well known, there may, under certain circumstances, be present in the throats which are apparently quite healthy virulent bacilli, which are capable of causing diphtheria in other patients."

The results of bacteriological examinations of scarlet fever patients on admission into hospital.

(1) *Throat.*

Pugh (p. 295) examined bacteriologically the throats of 420 unselected cases admitted into the North Eastern Hospital, certified as scarlet fever, of which two proved to be uncomplicated faucial diphtheria. From the throat of a patient suffering from scarlet fever and considerable obstruction to respiration, and from the throats of two others suffering from fibrinous rhinitis, virulent diphtheria bacilli were obtained. From 17 (4·5 %) of the remaining 415 cases diphtheria bacilli were cultivated, and five of these strains were tested for virulence with negative results, although they all stained with Gram's and Neisser's solutions and rendered neutral litmus glucose broth acid.

Garratt and Washbourn (1899) examined the throats of 666 cases of scarlet fever patients admitted under their care at the London Fever Hospital from March 1896 to December 1898, and found bacilli morphologically resembling *B. diphtheriae* in eight (1·2 %). Goodall (1896) found long diphtheria bacilli in the throats of six (7 %) out of 87 scarlet fever patients.

(2) *Nose.*

Pugh (p. 303) examined by cultures the noses as well as the throats of the 420 scarlet fever patients previously mentioned.

Diphtheria bacilli were obtained from the nose of one of the two diphtheria patients, and from the noses of the two cases of fibrinous rhinitis. Virulent diphtheria bacilli were also obtained from the nose, but not from the throat, of a child whose two sisters were suffering from diphtheria.

The remaining 414 cases presented on careful inspection no evidence of either faucial diphtheria or fibrinous rhinitis; nevertheless from the nasal cavities of 33 morphologically typical diphtheria bacilli were obtained. In 10 of these cases the organisms were present also in the throat, but in 23 they were present in the nose alone. Three of these strains were tested on animals and all were found to be non-pathogenic.

These observations show that diphtheria bacilli are frequently present both in the throats and noses of scarlet fever patients admitted into the London fever hospitals. In the case of those who show clinical evidences of faucial diphtheria or are suffering from fibrinous rhinitis the bacilli are generally virulent, but many of the bacilli obtained from patients without diphtheritic lesions are non-pathogenic to animals, and are probably incapable of giving rise to diphtheria.

The spread of diphtheria in scarlet fever wards.

The observations of Müller (1896) and others have shown that diphtheria bacilli rapidly become disseminated amongst patients in scarlet fever wards and amongst convalescents from this disease, when a patient suffering from diphtheria is introduced amongst them.

Chronic rhinitis with sore nostrils, a varying amount of discharge, and a tendency to the formation of pustules on parts of the body, is a fairly common sequel of scarlet fever. Todd (1898) called attention to the fact that this form of "external rhinitis" is sometimes due to the diphtheria bacillus (see p. 360).

Pugh (p. 305) says that "in similar cases at the North Eastern Hospital it is not uncommon to find that the condition is, in reality, fibrinous rhinitis," and gives an interesting account of an outbreak. "In a convalescent scarlet fever ward, occupied chiefly by girls of five, a child who developed rhinorrhoea was found to have membranes on the septum and the roof of the bony orifice of each nasal cavity; on the left tonsil was a small area of deposit. Cultures showed the presence of diphtheria bacilli in the throat and nose. The bacilli from one nostril were found to produce a strongly acid reaction in sugar broth, and 2 c.c. of a 48 hour broth culture proved lethal to a guinea-pig at the end of the second day, the result being prevented in another guinea-pig by the simultaneous injection of antitoxin. In three rounds of cultures made from the other 25 patients, who were kept in bed meanwhile, no fewer than 10 were found to have acquired the diphtheria bacillus. The spread of infection appeared to have been assisted by the fact that among the toys of the ward were school slates which were used indiscriminately by all. Well marked fibrinous rhinitis was present in two; their throats were normal, although, in one, diphtheria bacilli were present there also. The other eight children had diphtheria bacilli in the throat, which presented no abnormality except in one case, where there was a thin sheet of membrane on the right tonsil. In two the organisms were present in the nasal cavities also; these clinically appeared normal. Bacilli from each of the cases of fibrinous rhinitis, and from one of the healthy throats, were found to render glucose broth acid, and 48 hour broth cultures in 2 c.c. doses proved fatal to guinea-pigs in 48 hours, while in each case this effect was prevented in control animals by antitoxin."

Williams (1901) also found diphtheria bacilli in about 22 % of cases of post-scarlatinal rhinorrhoea. Of the 12 typical diphtheria bacilli tested for virulence four were found to be virulent and eight non-virulent.

The prevention of post-scarlatinal diphtheria.

This subject divides itself naturally into two parts, (A) the prevention of the introduction of virulent diphtheria bacilli into a ward, and (B) the prevention of the spread of the organisms amongst the patients.

A. *The prevention of the introduction of diphtheria bacilli.*

(1) *By members of the staff.*

"It follows from the observations which have been recorded that the transference of nurses from the diphtheria to the scarlet fever side of a hospital should not occur more frequently than can be helped, and that those who have been working in wards containing diphtheria or post-scarlatinal diphtheria patients should not be put on duty in scarlet fever wards unless they have been proved by culturing to be free from the means of infecting their charges with diphtheria."

A likely means both of acquiring and of distributing infection would seem to be the fondling and kissing of children; the rule, understood, in every hospital, that no child should be kissed by a nurse is without doubt very frequently broken.

(2) *By patients.*

A careful inspection of the throat on admission, with bacteriological examination in cases of doubt, is customary at all fever hospitals. That a similar examination of the nasal cavities is equally important is evident from some of the cases which have been quoted.

The important question arises as to whether a routine bacteriological examination of all patients should be made on admission. Such an examination of the throats was advocated by Garratt and Washbourn (1899) as a method of preventing post-scarlatinal diphtheria. During a period of 27 months from March 1896 to December 1898 they examined bacteriologically the throats of all the scarlet fever patients admitted under their care at the London Fever Hospital, and isolated all those in whom diphtheria bacilli were found, and by this means obtained a great reduction of the number of cases of post-scarlatinal diphtheria. The following table gives the proportion of such cases at the London Fever Hospital and in the Hospitals of the Metropolitan Asylums Board from 1893 to 1897.

	London Fever Hospital			Hospitals of the Metropolitan Asylums Board		
	Scarlet fever admissions	Diphtheria admissions	Post-scarlatinal diphtheria	Scarlet fever admissions	Diphtheria admissions	Post-scarlatinal diphtheria
1893	764	5	·52 %	14,548	2,848	1·40 %
1894	294	25	·34 %	11,598	3,666	1·90 %
1895	516	52	2·71 %	11,271	3,635	3·62 %
1896	637	65	·47 %	15,172	4,508	4·62 %
1897	431	45	·23 %	15,241	5,673	5·22 %
1898	325	40	0 %			

In consideration of the excellent results obtained by these observers it would seem advisable to examine the throats and noses of all cases on admission at times when diphtheria is prevalent, and to place all those found to be infected in separate wards. By this means the probability of the introduction of diphtheria amongst the mass of scarlet fever patients would be greatly lessened.

B. *The prevention of the spread of diphtheria bacilli amongst scarlet fever patients.*

When the virulent diphtheria bacillus has invaded a scarlet fever ward, as evidenced by the occurrence of a case of secondary diphtheria, the same measures as are applied in outbreaks of the disease in schools, etc., are indicated. "All the patients should be kept in bed, and inter-infection by their toys, handkerchiefs, etc., prevented, while the condition of their throats and nasal cavities is investigated, two rounds of cultures, at least, being made from nose and fauces. Similar cultures should also be made from the nurses. Those in whom diphtheria bacilli are found should be removed to an isolation ward and appropriately treated. When this method is adopted, a ward can usually be safely considered free from infection after only a few days quarantine.

Attention has been directed to the importance of an examination of the nurses and of a supervision over the toys (especially with regard to the slates and mouth instruments, so frequently supplied by parents), because in several examinations in our wards, in connection with cases of post-scarlatinal diphtheria, these have appeared to be important factors in stamping out infection" (Pugh, p. 311.)

Large many-bedded wards and common recreation rooms tend to favour the spread of secondary diphtheria, since infected children are in both cases brought into intimate contact with numbers of more or less susceptible patients.

"The possible harm which may be done by the introduction of virulent diphtheria bacilli into a ward is to be gauged, not by the number of patients who develop post-scarlatinal diphtheria, but by the number infected by the bacillus. The former, which alone is recorded in a hospital's statistics, is no guide to the amount of evil which may possibly result from the discharge to their homes and schools of children who, though apparently healthy, carry with them the virulent bacillus of diphtheria."

For this reason the use of prophylactic doses of antitoxin in patients exposed to infection without adequate bacteriological examination, as suggested by W. R. Smith (1900), cannot be recommended, since it encourages a free distribution of virulent bacilli amongst the protected patients, who on their discharge may work havoc amongst others.

CHAPTER XIII.

DIPHTHEROID ORGANISMS IN THE INSANE.

The occurrence of diphtheroid organisms in general paralytics in the mouth, nose, genital organs, and internal organs. Characters of the organisms. Eyre and Flashman's observations. Diphtheria bacilli in the tissues.

WITHIN the last few years Robertson and his collaborators have brought forward evidence to show that diphtheroid organisms, which they consider to be non-virulent diphtheria bacilli, are to be found in the tissues of patients who have died of *General Paralysis of the Insane.* They have also demonstrated similar organisms in certain situations in living persons suffering from various stages of the disease. As a result of their observations, Robertson, McRae and Jeffery (v. 1903) "have advanced the hypothesis that general paralysis is the result of a chronic toxic infection from the respiratory and alimentary tracts, permitted by the general and local impairment of the defences against bacteria, and dependent upon the excessive development of various bacterial forms, but especially upon the abundant growth of a diphtheroid bacillus, which gives the disease its distinctive character" (Robertson, 24. x. 1903, p. 1065). Though the final verdict on the relationship of this organism to the disease must rest on the evidence of more extended observations, the results of the experiments already made are extremely interesting and important, and are briefly summarised below.

Although previously to 1903 several observers had investigated bacteriologically the blood, cerebro-spinal fluid and urine of general paralytics, none had met with organisms which they thought bore any relation to the disease. Early in 1903 Robertson, McRae and Jeffery (v. 1903) published a detailed account of their observations on the secretions, blood and tissues of general paralytics. Further contributions on the same subject have been published by Robertson (VII. 1903, 24. X. 1903, and 25. X. 1905), Robertson and McRae (V. 1905), and Robertson and Shennan (IV. 1903), on which the following account is based.

The occurrence of diphtheroid organisms in general paralytics.

(1) *In the mouth and throat during life.*

"We have succeeded in obtaining cultures of the Klebs-Loeffler bacillus from the tonsils, pharynx, carious teeth and saliva of cases of general paralysis during life, and have also observed the organisms in considerable numbers in film preparations made directly from material from their surfaces. In the only instance in which we have been able to obtain expectoration for examination the Klebs-Loeffler bacillus was found to be present in enormous numbers."

In many other instances, however, they failed to find diphtheria-like bacilli in these situations, and Robertson clearly states that he does "not attach any special importance to the occurrence of the organisms in the throat."

(2) *In the genital organs during life.*

Robertson and McRae (V. 1905) state that apparently all female genital paralytics suffer from leucorrhoea (36 consecutive cases). In all 14 of such cases bacteriologically examined he obtained diphtheroid organisms in smears and in cultures, and he also found them in material taken from the surface of the urethra of 22 consecutive cases of general paralysis in the male.

(3) *In the blood during life.*

Nine observations were made of the blood during life, but diphtheroid organisms were never found.

(4) *Cultures from the tissues after death.*

Twenty cases altogether were examined as recorded in the first paper (V. 1903), and from the tissues of 17 diphtheroid bacilli were obtained in cultures and "in the three from which they were not isolated they were readily found in microscopic preparations of the stomach in two of the cases and in a film preparation of the material on the surface of the trachea in the third."

The following table shows the number of times the organisms were isolated from different situations.

Situation	Cases examined	No. of cases in which diphtheroid organisms were isolated
Tonsils or pharynx	12	9
Bronchi	5	3
Lung tissue	6	3
Stomach	16	7
Inflamed portion of ileum	16	5
Brain	16	4
Bone marrow	5	0

In two later examinations Robertson and McRae (V. 1905) obtained these organisms from patches of localised purulent cerebral meningitis in two cases of general paralysis. In one case they were associated with *B. coli* and in the other with pneumococci.

"The tonsils were almost constantly found to contain small purulent foci on section, and it was chiefly from these that the growths of the diphtheria organism were obtained."

The method of obtaining the cultures from the stomach and intestines was as follows: "Cultures were made from the submucous or mucous coat of the small intestine and stomach, after searing the outer surface and incising the peritoneal and muscular coats with a sterile knife, from the inner surface of these organs."

These cultivations were made on byno-haemoglobin agar.

(5) *Histological evidence of the presence of diphtheroid organisms in the tissues of general paralytics.*

Robertson (VII. 1903) studied the morbid alterations in the alimentary tract of 40 cases of general paralysis, and sought for the presence of the diphtheroid bacillus in 20. Chronic catarrhal changes of a severe nature were constantly found, either in the stomach or small intestines, and frequently in both.

The respiratory tract was studied in five cases. For the purpose of identifying the bacilli he used a modification of Neisser's method, and Weigert's modification of Gram's method. Working with these and other methods he was able "to recognise in the catarrhal exudations in the alimentary or respiratory tracts of all the 20 cases investigated, a bacillus identical in form and staining reactions with the organism isolated by culture methods. In most of the cases the bacilli were to be observed in considerable numbers, and in eight they were present in very great numbers. In these eight cases their ascertained distribution was as follows:—In the stomach in three cases; in the stomach and lower part of the ileum in one case; in the crypts and surface of the tonsils in two cases; and in the bronchi and stomach in two cases. The organism was found in the respiratory tract in each of the five cases in which this region was examined."

He states that in one of his cases the bacilli were so abundant in the lower respiratory tract that in a transverse section of one of the bronchi stained by Gram's method, in which microscopic examination proved that only those organisms had retained Gram's stain, a broad violet ring lying on the surface of the mucous membrane could be seen with the naked eye.

He also observed a filamentous organism in the lymphatics of the walls of the alimentary and respiratory tracts. "This organism has been observed in enormous numbers in five of the 20 cases investigated, namely, in two cases in the wall of the stomach alone, once in the stomach and ileum, once in the ileum alone, and once throughout the whole extent of the bronchi and trachea." "This organism appears as smooth threads of various lengths, and, as seen in carbol-thionin preparations, shows alternate pale and dark portions, the latter being shorter. Bacillary forms are common." The author gives some reasons for thinking that the organism just described may be a streptothrix form of the diphtheroid organism.

(6) *Characters of the organism.*

The organism grows well on serum and byno-haemoglobin agar producing colonies identical with those of the diphtheria bacillus. Morphologically it is indistinguishable from the diphtheria bacillus and shows well marked polar granules by Neisser's method. Acid is rapidly produced in glucose broth. The other cultural characters have not been described.

Guinea-pigs are not affected by subcutaneous or intraperitoneal injections. Intrapleural injection into a white rat caused death in five days, and "microscopic examination of the tissues showed that the organism had multiplied at the seat of injection and spread into the adjacent pulmonary tissues and into the pericardium." Three rats were fed with bread mixed with broth cultures and remained well for three or four weeks. After this time they began to show morbid symptoms which increased until they became acutely ill. At first they showed slowness and unsteadiness in gait and drowsiness after feeding. "Later they manifested distinct motor weakness, marked incoordination of movement, dyspnoea, great drowsiness, and looseness of their motions." One was killed when moribund and the others died. Control animals under identical conditions remained healthy.

Microscopical examination of these rats revealed a series of pathological changes, differing in individual cases only in degree. Gastro-intestinal catarrh, localised particularly at the upper part of the intestine, proliferative and degenerative changes of the liver, and inflammatory changes of the lungs were found. The most interesting changes were observed in the brain and consisted of extensive degeneration of a large proportion of the cortical nerve cells, early acute periarteritis, proliferation of the neuroglia and mesoglia cells, and infiltration of the pia-arachnoid.

During the course of several months Dr C. L. Bruce from time to time subcutaneously inoculated a goat with cultures of this diphtheroid organism in order to obtain an immune serum. After a time the animal developed signs of a severe alimentary disturbance. "It became tottering in its gait and about six months from the time the last subcutaneous injection had been made it had a seizure closely resembling the congestive attack of a general paralytic. It rallied to some extent, but died a few days later." Cultures of the diphtheroid bacillus were obtained from the oesophagus, and the brain showed changes resembling those found in general paralysis.

Robertson and Shennan (1903) summarise the results of their animal experiments as follows:—"Whatever the relation of this organism may be to general paralysis of the insane, the evidence we have obtained distinctly shows: (1) that when introduced by way of the alimentary tract in the form of broth cultures, it is capable of producing in the rat a series of morbid phenomena, which especially affect the nervous system, and which when once established may go on progressively till death results, even though the feeding with cultures is stopped; and (2) that the associated changes in the central nervous system have a distinct resemblance to those which occur in dementia paralytica."

Some of the work of the investigators, who have just been quoted, was repeated by Eyre and Flashman (1905). They examined by means of cultures on serum material from the throats of 138 insane patients, of whom 60 were suffering from general paralysis, and 78 from other forms of insanity, and found the diphtheria bacillus in seven, and

Hofmann's bacillus in 14. The diphtheria bacilli occurred in the throats of three (5 %) of the 60 general paralytics.

The injection into guinea-pigs of small quantities of living cultures or of the filtered products of two of these three strains was without effect. One of these strains, but not the other, was capable of killing guinea-pigs when the living bacilli were injected in large doses.

A series of examinations by means of cultures was also made from material derived from autopsies on 10 cases of general paralysis and 26 cases of other forms of insanity. Swabbings from the pharynx and bronchi, scrapings from the mucous membrane of the intestine, and specimens of the cerebro-spinal fluid, heart's blood and bile were obtained. Diphtheroid bacilli were never found in the cerebro-spinal fluid, or the bile. In the examinations of general paralytics diphtheroid bacilli were found in four out of seven cultivations from the pharynx, three out of ten from the bronchi, one out of eight from the heart's blood, and one out of ten from scrapings from the intestinal mucosa. No further investigation of these organisms appears to have been made. Diphtheroid bacilli were also met with on four occasions in the respiratory tract in 26 examinations of patients suffering from other forms of insanity.. The authors are satisfied that not one of the organisms isolated from post-mortem material was *B. diphtheriae* (Eyre, 9. XII. 1905, p. 1558).

As a result of their experiments these authors came to the conclusion that they were "unable to trace any causal connection between *B. diphtheriae* and general paralysis of the insane," and suggested that Robertson and his colleagues were dealing with a "local or Asylum infection."

Local conditions might undoubtedly account for the high percentage of infection in various parts of the buccal cavity observed by Robertson, and the fact that diphtheria-like organisms are to be found both in vagina and male urethra has been pointed out by several observers (see p. 378). The extraordinary percentage of infection of the genito-urinary organs in Robertson's series, however, suggests that the presence of these organisms was more than accidental. His observations on their occurrence in large numbers and in every case in the inflamed portions of the gut, their frequent occurrence in the brain and their action on animals, all point to their connection with the disease, and render further investigations desirable. Eyre (9. XII. 1905) states that in his ten cases no catarrhal areas were discovered in the intestinal canal, although a careful search was made in each case. Eyre and Flashman (1905) only examined the cerebro-spinal fluid and not the brain, and appear to have made no animal experiments comparable with those of Robertson and Shennan.

In connection with this subject certain records of the finding of diphtheria-like bacilli in the brain and blood, and in the crypts of the tonsils of persons who had not suffered from diphtheria, and certain observations on the fate of diphtheria bacilli in the alimentary tract, are of interest.

Johnston and Goodall (1902) cultivated from the heart's blood, but not from the cerebro-spinal fluid, of a woman, who died in "an acute confusional state, possibly a phase of dementia paralytica," a diphtheria-like bacillus, whose action on animals was not tested.

Head and Wilson (1899) cultivated diphtheria bacilli, virulent for guinea-pigs, from the brain of a suspected case of rabies, and Morrell and Wolf (1906) twice isolated virulent diphtheria bacilli during life from the cerebro-spinal fluid of a child suffering from general miliary tuberculosis and tubercular meningitis. Although no signs of diphtheria were present diphtheria bacilli were also found in the throat. Howard (1894) isolated non-virulent diphtheria bacilli from the heart valves of a patient who died of acute ulcerative endocarditis.

Marzinowsky (1900) examined by means of cultures both the surfaces and the crypts of the tonsils of 15 persons, who had died from various diseases.

Non-virulent diphtheria-like bacilli, retaining Gram's stain and showing polar granules by Neisser's method, were isolated from the crypts in six cases (20 %), but only in one instance from the surface of the tonsil. In two other cases (pneumonia, anthrax) virulent diphtheria bacilli were found in the crypts. Schütz appears to have isolated both attenuated diphtheria bacilli and diphtheria-like bacilli from the sputum of tuberculous patients.

Although several examples of diphtheritic lesions of the stomach have been recorded (p. 97), but little attention has been paid to the fate of diphtheria bacilli in the intestinal canal. Süsswein (1902) has recorded a few observations on this subject. In 15 cases of diphtheria examined during life, he was unable to detect the diphtheria bacillus in either the gastric or intestinal contents. At the autopsies on eight cases he was able to detect the diphtheria bacillus in films from the gastric contents in four cases, but was able to obtain cultures in two cases only. He never observed it in the jejunum. Diphtheria bacilli kept at 37° C. in acid "stomach contents" died within an hour, but neither neutral "stomach contents" nor the contents of the gall-bladder affected them.

Schoedel, J. (1900) apparently observed diphtheria bacilli both in the lower ileum and in the freshly passed faeces of persons suffering from diphtheria.

SECTION V.

CHAPTER XIV.

THE TYPES OF IMMUNITY.

BY G. DEAN.

Introduction and definition. "Virulence and toxicity." Natural immunity. (*a*) Natural immunity in the individual, (*b*) Racial immunity, (*c*) Natural immunity in the species. Protective mechanism at the usual sites of diphtheria infection. Acquired immunity, active and passive. Inherited immunity.

Definitions and Introduction.

IMMUNITY may be defined as a condition of natural or acquired resistance possessed by individuals, races or species of animals to noxious influences which, in the absence of this refractory state, are capable of producing disease. The most important of these noxious influences are the animal and vegetable parasites, and chief among these the bacteria and their toxins.

Immunity is the expression of the reaction of the organism by which it attains partial or complete protection, and this varies according to the nature of the attack. In approaching the question of immunity, therefore, in any particular bacillary disease one of the first points for consideration is the method of attack of the invading microbe.

Though the pathogenic bacilli produce their effects on the animal organism chiefly by their toxins, the manner of the attack may vary greatly in the different groups of microbes. Diseases can be roughly classified according to the mode of action of the causal pathogenic micro-organisms, and such a classification is of the highest importance from the point of view of immunity.

One group of micro-organisms remains localised at or near the point of invasion. Here they elaborate their toxins, which are absorbed along the lymphatics, blood vessels or nerves, and find their way to and attack that part of the body for which they have a selective affinity. In such cases the predominant feature of the disease is the toxic action and the condition is known as a "toxaemia." Diphtheria and tetanus may be taken as types of this toxaemic form of disease.

In another group of bacterial diseases the microbe, having found an entrance into the animal body, does not remain localised but invades the blood, organs, and tissues of the body and multiplies in them. Anthrax and streptococcal septicaemia may be taken as types of this septicaemic group. There are other diseases which occupy an intermediate position between the two groups; of these typhoid and Malta fever may be mentioned as examples.

The organisms giving rise to the two types of disease differ from each other in one important respect. While those which cause the toxaemic type, when cultivated, freely secrete their poisons into the nutrient media, the others do not secrete soluble toxins. In fact it is difficult or impossible to obtain the toxins apart from the bodies of the latter bacteria.

The mechanism of immunity in the two groups is very different. In both cases the body strives to get rid of the invading parasite, but in the one group the chief reaction is directed against the toxic substances, which are being poured into the body; in the other group, the chief forces of resistance are directed against the microbes themselves.

The Terms "Virulence" and "Toxicity."

The *virulence* of a microbe is the power which it possesses of attacking, invading and breaking down the defences of a susceptible animal, thus giving rise to disease or death.

Toxicity is the power of a toxin to produce disease or death in a susceptible animal. The measure of the virulence or toxicity is inversely proportional to the quantity of living culture of the microbe or of its toxin required, under standard conditions, to produce a certain pathological effect; in the case of diphtheria, death within four days.

The toxicity of a culture or toxic filtrate is largely dependent on the toxigenicity of the race of micro-organism employed. The toxigenic property must be sharply differentiated from the virulence. The virulence of a micro-organism is no doubt largely due to its power of producing toxin within the animal body, but the toxigenicity of a particular microbe, as ascertained by measuring the toxicity of culture filtrates, cannot be taken as a true index of virulence. A race of diphtheria bacilli may have the property of producing toxin on artificial media very highly developed, and yet when introduced into the animal body may be less capable of producing pathological changes than a race with very low toxigenic properties.

NATURAL IMMUNITY.

Definition.

The property of resistance or insusceptibility possessed naturally by individuals, races, or species of animals to certain diseases is termed "natural immunity."

Examples of Natural Immunity with Remarks as to Mode of Origin and Causes.

(*a*) *Natural immunity in the individual.*

That certain individuals prove less susceptible than others when submitted to the same risk of infection is a matter of common knowledge. It is generally admitted also that an infective disease proves more serious to certain individuals than to others of apparently equal physical endurance. In cases of food poisoning, where a number of persons partake of about the same amount of infected material and thus get approximately the same dose of the infective germs, it is not an uncommon experience to find that one or two individuals of a series of say twenty, prove almost or altogether refractory to the infection. The causes of such immunity in individuals can only be surmised. Reference will be made to the subject in speaking of the question of antitoxin in the blood of normal individuals (p. 453).

(*b*) *Racial immunity.*

That the processes concerned in evolution are at work in the production of racial immunity, as in every other domain of biology, cannot be doubted. The benign character of certain diseases, such as measles, as they occur in an old established habitat among Europeans compared with their malignancy when imported into fresh regions, such as the

Sandwich Islands, can be most readily explained on the hypothesis of an immunity evolved by attacks of the disease affecting many generations. The dark races in Brazil are more refractory to yellow fever than the Europeans. How far these, and such similar examples as malaria, may be due to increased resistance as the result of evolution, and how far to an immunity acquired by the survival of the individual from an attack during childhood is unknown.

An excellent example of racial immunity among the lower animals is the comparative insusceptibility of the Algerian sheep to anthrax, one of the most deadly diseases to other races of sheep.

(c) *Natural immunity in the species.*

Metchnikoff (1901) in his review of the animal kingdom from the point of view of immunity found that most of the lowest forms of animal life were insusceptible to the action of diphtheria toxin. Among the insects he found, however, that while certain species were highly susceptible, other species, for example the larvae of a beetle (*Oryctes nasicornis*), manifested a marked immunity to the diphtheria bacillus and its toxin. The alligator (*A. mississippiensis*), while refractory to tetanus toxin, was found to be highly susceptible to diphtheria toxin.

An animal may possess a natural immunity to a bacillus, or to its toxins or both, but the possession of the one property does not necessarily imply the possession of the other. This distinction between immunity to infection and immunity to intoxication must be insisted on.

The rat and the mouse may be taken as examples of the lower animals possessing a natural immunity to diphtheria. These animals are capable of supporting very large doses of living cultures, or of toxin, when injected subcutaneously or intravenously. This immunity is not absolute, for, although the rat will support a dose of toxin containing many multiples of the lethal dose for the guinea-pig, by massive doses this resistance may be broken down, as was shown by Cobbett (1899) and others. That this natural immunity of the rat is not due to the presence in the blood of substances of the nature of antitoxins was demonstrated by Kuprianow (1894) who mixed rat's serum with toxin and found that the latter was not neutralised as evidenced by tests on guinea-pigs. Behring (1899) suggested that such cases of natural immunity are due to a physical cause, an impermeability of the walls of the capillaries which hinders the toxins from readily reaching the sensitive cells. Behring holds, however, that in certain cases one must assume a cellular immunity. But it can no longer be maintained that the immunity is in all cases due to an insusceptibility of the cells, for Roux and Borrel (1898) have shown that when the toxin is introduced directly into the cerebral substance it causes a paralysis, which may last several days and prove fatal. The toxin therefore, according to these authors, is innocuous not because the brain cells are insusceptible but because the toxin does not reach them. According to Metchnikoff this is due to the fact that the leucocytes set up a barrier which prevents the toxin from reaching the sensitive elements. The immunity of the rat and mouse may be explained in two ways from the standpoint of Ehrlich's side-chain theory. Either the animal may be wanting in the necessary side-chains by means of which the toxin is anchored to the sensitive cells, or the sensitive cells with suitable receptors may be distributed widely throughout the animal body, and in that way the force of the toxin may be diffused and diverted from organs necessary to the life of the animal. As a matter of fact no very satisfactory explanation has been given of the great differences which exist amongst the various species of animals in their susceptibility to toxins. In the chapter on diphtheria toxin will be found a statement of its relative toxicity for different species.

The question arises as to how far in the human subject the resistance possessed by certain individuals may be due to the presence of antitoxin in the blood. That antitoxin frequently occurs in the blood of healthy individuals has been shown by a number of observers, among them Abel (1894) and Wassermann (1894–5). The frequent presence of diphtheria bacilli in the throats of apparently healthy individuals (see p. 181) would yield a satisfactory explanation in the case of the human subject. Wassermann came to the conclusion that the occurrence of the antitoxin was due to the presence of "pseudo-diphtheria bacilli" in these cases. He thought that these organisms might produce small quantities of toxin sufficient to stimulate the antitoxin-producing mechanism, and yet not powerful enough to produce toxic effects. An observation by Lubowski (1900) is interesting in this connection. He isolated from a child a bacillus which proved to be avirulent and atoxic, and yet the serum of the child was found to be highly antitoxic. We must, however, distinguish between avirulent diphtheria bacilli and the bacillus of Hofmann. Petrie (1905) found that the latter was unable to produce any substances capable of giving rise to diphtheria antitoxin when injected into the horse.

Metchnikoff (1901) has suggested a mechanism which may account for the antitoxic properties of the blood serum of the hedgehog, snake, mongoose, and scorpion, namely, that these animals in fighting and catching their prey must frequently be subjected to slight abrasions with resulting absorption of small doses of toxin thus giving rise to the production of antitoxin.

It is more difficult to explain the presence of diphtheria antitoxin in the normal horse for here diphtheria bacilli are probably of rare occurrence. In one case only has their presence been definitely proved (Cobbett, 1900). Ehrlich explains the occurrence of antitoxin in the serum of normal animals by supposing that the antitoxin exists normally in a preformed state in the cells as the receptors which have a specific affinity for the toxin, and that some of these get thrown off into the blood serum. This subject will be dealt with more fully in discussing Ehrlich's theory.

Animals, which on account of their susceptibility to infection and intoxication are selected by preference for experiment, such as the guinea-pig and mouse in the case of anthrax and tetanus, and the guinea-pig in the case of diphtheria, may yet be regarded as immune to these diseases from the epidemiological, "epizootic" standpoint, for the occurrence of tetanus, anthrax and diphtheria in these animals under natural conditions is unknown. As Behring (1901, p. 95) has pointed out, some confusion may arise if the distinction between these two forms of immunity, the epidemiological and the experimental, is overlooked.

Protective Mechanism at the Usual Sites of Diphtheria Infection.

When we consider the number of organisms, often including pathogenic forms, such as the diphtheria bacillus, which find their way into the nose and mouth, it becomes clear that there must be at work an effective mechanism which prevents the settlement and growth of the invaders. That the conditions found in the alimentary canal from the mouth downwards are not unfavourable to microbial life is shown by the large number of non-pathogenic bacilli which can be cultivated from almost any part of its course, and as the pathogenic bacilli are not less resistant than the saprophytes we must assume that the conditions are favourable to many of them. What are the factors then which tend to prevent such pathogenic bacilli as the *B. diphtheriae* from attacking the individual? It is doubtful whether the antagonism between pathogenic and non-pathogenic bacilli plays an important part. Certain feeding experiments carried out by Metchnikoff (1894) and Wiener (1896) indicate that such an antagonistic action may occur. So long as the kittens and young rabbits, which were employed in the experiments, were being suckled by the mother they could be infected by certain vibrios *per os*, but as soon as they were put on a mixed diet infection could no longer be accomplished. The converse fact that non-pathogenic bacilli may aid the pathogenic has, however, been proved by many observers.

The epithelial layers, including the ciliated nasal epithelium of the mucous membranes, form an important barrier to the attack of microbes, and the constant shedding of this epithelium must be of great service in overcoming surface invasion. The secretions, mucus and saliva in the mouth, mucus in the nose, and tears in the conjunctival sac and nasal duct, have an important mechanical cleansing action. Particles of dust, etc., loaded with microbes, alighting on the sensitive mucous membrane cause an increase in the secretions which tends either to wash away the foreign substances, or by accumulating to give rise to the stimuli necessary for the reflex actions of coughing or sneezing, or the voluntary clearing of the nose or throat, all of which lead to the ejection of a large number of microbes. The muscular movements connected with the mouth, fauces, palate, and posterior nares must also aid in a mechanical way. The evidence as to whether the fluids concerned have bactericidal properties is somewhat conflicting. Wurtz and Lermoyez (1893) found the nasal mucus bactericidal, an opinion which was controverted by Klemperer (1896). Thompson and Hewlett (1896), though they found that the mucus was not bactericidal, proved that the nasal mucus arrested bacterial development and that there was a rapid disappearance from the nasal cavities of artificially introduced bacilli such as *B. prodigiosus.* In regard to the saliva Hugenschmidt (1896) found no bactericidal action, whereas Sanarelli (1891) found that it had bactericidal properties. As pointed out by Miller (1892) the evidence relied on by Sanarelli was insufficient. The important function exercised by the lymphoid tissues of this region has been urged by Metchnikoff, who regards them as manufactories of phagocytes. Stöhr (1884) made the important observation that the leucocytes wander out of the tonsils, and amongst others Ribbert (1885), Bizzozero (1885), Manfredi (1886), and Ruffer (1890) have shown that the cells of the lymphoid organs may contain bacilli. Every one who has examined the nasal secretion in pathological conditions must have been struck by the frequent occurrence of leucocytes containing bacilli. It is probable that the serum, though present only in minute traces in the secretions, may assist in preparing the bacilli for phagocytosis, and so play an important part in the defence of the body. Hugenschmidt (1896) attributes the protection of the mouth against invasion to phagocytosis aided by the positive chemiotactic character of the saliva. He maintains that diapedesis is a very marked feature in the mouth, and states that the sulphocyanide of potassium to which antiseptic properties had been ascribed has no such properties, and even doubts the existence of such a substance in saliva.

In the protection of the lungs several of the factors already mentioned play a part. That the phagocytes are as important here as elsewhere is probable. Hildebrandt (1888) found anthrax bacilli in the dust-cells, "Staubzellen," which he regards, however, as cast-off epithelial cells and not as macrophages, as held by Metchnikoff.

The general conditions which predispose to infection will be dealt with in another section. It is well, however, here to recall how susceptible the diphtheria bacillus is to slight changes in its surroundings, e.g., we shall see how the presence of an excess of sugar or a slight change in reaction of the culture medium can prevent toxin formation. Such considerations enable us to understand how very slight alterations in the animal body may have an important bearing in determining whether the bacillus will succeed or fail in obtaining a hold on the individual subjected to infection.

ACQUIRED IMMUNITY.

A susceptible animal may during its life acquire an immunity to a disease; in many cases an increase in natural resistance occurs as the animal grows older. From the earliest times it has been a matter of common observation that those who survived an infectious disease such as smallpox, syphilis, etc., acquired an immunity to that disease, in other words that recovery from a disease may do away with the natural proclivity to that disease. Where such an immunity is acquired by the survival of the individual from an attack of infective disease it may in a sense be regarded as "natural." It is Nature's way of conferring immunity, and the recognition of this fact has been the chief guiding principle in the study of the subject. On the other hand, where the immunity is acquired through the intervention of man by the employment of some process of inoculation, the immunity is termed "artificial." An artificial immunity may be conferred by the use of a bacterial or other vaccine, or by the use of an immune serum, and to designate these two conditions Ehrlich introduced the terms "active" and "passive" immunity, which may be thus defined—

Active immunity is the immunity which an animal acquires as the result of treatment with bacilli, toxins, or other substances, which may be of bacterial, vegetable, or animal origin.

Passive immunity is the immunity which results when an animal is injected with the antitoxin or other antibody, which has been obtained by the active immunisation of another animal.

In active immunity the immunised animal manufactures its own antibody. The immunity acquired by the horse as the result of injections of toxin may be taken as an example of active immunity. In passive immunity the immunised animal obtains its antibody ready made from another animal. The immunity conferred on a child by an injection of diphtheria antitoxin will serve as an example of passive immunity.

Behring (1899) takes exception to the terms "active" and "passive" immunity (p. 997). He says one can speak of an "active" or "passive" immunisation but he prefers to use the terms "isopathic" and "antitoxic" immunity. In both active and passive immunity he says that the essential thing is a haematogenous immunity which is due to the presence of antitoxin in the blood. It has still to be proved that there exists, in addition to this haematogenous immunity, a histogenous immunity due to an altered condition of the organised parts of the body, a view to which he formerly leant. There is, however, he admits, no sure experimental proof of the existence of an acquired histogenous immunity even in the case of an isopathic immunity, and it is now generally agreed that after the injection of antitoxin the living organised tissues remain unaffected, i.e., no histogenous immunity results. The immunity which a young animal acquires from its mother is derived from the milk and not by the inheritance of an acquired cell change.

Behring believes that, instead of increasing in their resistance, the cells susceptible to the action of toxin undergo changes due to modified activity which may result in a hypersensibility. If a hyposensibility could be demonstrated to result from active immunisation it would definitely prove the existence of a histogenous immunity. Behring at first thought such a hyposensibility could be observed in horses immunised against tetanus, but further observations made him abandon this view, for when the same antitoxic value of the blood serum had been produced in two animals, in one by passive, and in the other by active immunisation, the injection of equal doses of toxin appeared to produce more serious effects on the actively immunised animal than on the other. This he regarded as evidence of a hypersensibility. The observation that a horse which has been actively immunised retains his immunity much longer than a passively immunised man, guinea-pig, rabbit, or goat cannot be employed as an argument in favour of an increased resistance of the cells, which before immunisation were sensitive to the action of a toxin, for it has been found that if the serum of the same species is employed, the antitoxin remains much longer in the body than if the serum of another species is injected (Behring, Ransom).

This, however, is not universally true, for Jörgensen and Madsen (1902), in their study of cholera and typhoid agglutinins, found that the rapidity with which the antibody disappeared depended on the animal employed, and that no general law could be established. They found, for example, that the agglutinating serum of the rabbit and goat disappeared at about the same rate from the goat, whereas different results were obtained with the two sera in the case of the rabbit. In the same paper they make what appears to be a fundamental distinction between active and passive immunity. The injection of typhoid and cholera organisms was followed both in the normal animal and in the actively immunised animal by a sudden increase in the agglutinin giving a curve of a very definite type, whereas in the animal passively immunised there was an absence of this typical rise in the curve. The injection of quite a small quantity of agglutinating serum into an animal was sufficient to abolish the typical reaction.

INHERITED IMMUNITY.

Acquired immunity, obtained by the action on the body of the virus, whether introduced by Nature in the form of an attack of the disease or by man in the form of a vaccine, or by the injection of the serum of an immunised animal, is as a rule a transitory property belonging to the individual only. In certain cases, however, the immunity can be transferred from the parent to the offspring and to such cases the term "inherited immunity" is applied. Such an inherited immunity might conceivably be conveyed by

(*a*) the transference from the parents to the offspring of the acquired property by means of the germ plasm, (*b*) active immunisation; the toxin acting through the body of the mother on the germ plasm or on the developing foetus,

(*c*) passive immunisation; the foetus obtaining the protective substance manufactured by the body of the mother through the placental circulation or from the mother's milk.

The results of the experimental investigation of the relative importance to be ascribed to these possible sources of inherited immunity will be dealt with in a later section.

CHAPTER XV.

THE TOXIN OF THE DIPHTHERIA BACILLUS.

The term "toxin." Discovery of diphtheria toxin. Toxicity of different races of diphtheria bacilli. Conditions which modify the virulence and pathogenic powers of the diphtheria bacillus (*a*) association with other germs, (*b*) passage through animals. Influence of the medium on the production of toxin. Influence of temperature, oxygen, and light on the diphtheria bacillus. The period of maximum toxicity in the cultures. Methods of testing the toxicity of the diphtheria bacillus. The relative susceptibility of different races of animals to diphtheria toxin. On the origin and nature of toxin. The constitution of the diphtheria toxin. Toxoids. The spectrum according to Ehrlich. Conditions which modify toxin, time, light, temperature, electricity, oxygen, peroxides and oxydases. Chemical agents. The purification and isolation of the diphtheria toxin. The effects of toxin on animals. Subcutaneous injection. Latent period. Local effect. General effect. Intracerebral injection. Physiological effects on the heart and circulation. The fate of the toxin *in corpore* (1) in susceptible animals, (2) in refractory animals, (3) behaviour of toxin in the alimentary canal, (4) action of toxin on other mucous membranes.

The Term "Toxin."

TOXIN, in the restricted bacteriological sense, may be defined as the specific poison secreted by a pathogenic micro-organism. By the specific poison is meant the substance giving rise to the chief pathological effects which taken together constitute the disease.

The true toxins can be recovered from the cadaver of a subject of the disease and also from the media in which the bacilli have been grown.

The bacilli from which such toxins have been obtained constitute a small group, the chief of which are *Bacillus diphtheriae*, *Bacillus tetani*, *Bacillus botulinus* and *Bacillus pyocyaneus*.

The action of a toxin on the animal body is characterised by (*a*) the occurrence of a latent or incubation period after the injection, during which no effect on the animal is apparent, and (*b*) the production of antitoxin.

The toxin is a "haptine," that is, a substance capable of anchoring onto certain structures of the body and stimulating them to the formation of an antibody.

We have no exact knowledge as to the chemical nature of toxins. Though generally associated with albuminous substances, they are not at the present time usually regarded as toxalbumins. They are unstable, being extremely sensitive to the action of light, heat and chemicals. In many of their characteristics they closely resemble the ferments.

The word toxin in the wider sense has been applied to various poisonous bodies elaborated by micro-organisms including the "ptomaines," or crystalline nitrogenous bases of definite chemical composition, the toxalbumins and the proteins, substances entering into the structure of the bacillary body, none of which merit the title of toxin in the narrower sense as they are not concerned in producing the specific effect of the bacillary action. Even such substances as tuberculin and mallein, though they produce certain specific reactions, can be spoken of as toxins only in a broad sense.

The term toxin is also applied to various poisons of animal and vegetable origin; as examples of the former may be mentioned the snake poisons, or venins, and the ichthyotoxin of eel's serum; of the latter such substances as ricin and abrin.

DISCOVERY OF DIPHTHERIA TOXIN.

Loeffler (1884) in his work on the diphtheria bacillus indicated that it was necessary to assume the existence of a toxin to explain satisfactorily the production of the disease by the bacillus. At a later period (1890) he was able to prove that a toxin could be obtained by growing the bacillus on minced beef, extracting with glycerine, and precipitating by alcohol.

To Roux and Yersin (1888, 1889, 1890) belongs the credit of first obtaining a diphtheria toxin. By methods which could easily be followed and repeated they obtained toxins which gave all the characteristic appearances of diphtheria poisoning. The essential point in the method consisted in growing the diphtheria bacillus on broth for several weeks at body temperature and filtering the culture through an unglazed clay filter. The toxicity of sterile filtrates from cultures 30 to 40 days old was such that guinea-pigs were killed in about 30 hours by doses of from $\frac{1}{8}$ to $\frac{1}{15}$ c.c. Though the toxins obtained were weak compared with those that can be obtained at the present time their action on animals, and the effects on the toxin of heat, chemical agents, dialysis, etc., were so accurately noted and described that the memoirs of Roux and Yersin on the subject must always rank as among the most noteworthy of the classics of experimental medicine.

Toxicity and Virulence of Different Races of Diphtheria Bacilli.

When cultures are made from cases of diphtheria the bacilli, grown under the same conditions on a medium suitable for toxin production, yield filtrates of very different degrees of toxicity. It cannot be said that there is any very marked correspondence between the severity of the disease in the human subject and the virulence or toxigenic power of the bacillus obtained from the case. Some of the most virulent and toxigenic races of bacilli have been derived from comparatively slight cases of diphtheria. A notable example of this is Park and Williams' bacillus No. 8 which was obtained from a mild tonsillar case. As a rule the most virulent bacilli yield the strongest toxins but not invariably. A bacillus which has a low degree of virulence may yield a stronger toxin than a highly virulent one (Behring (1901), Salter (1896)). Indeed, as shown by Martin (1898) a bacillus may be toxigenic and yet non-virulent. A process of selection, therefore, has been employed to obtain bacilli which yield powerful toxins. As the race of bacillus is, perhaps, the most important factor involved in obtaining good toxins, a great service was done by Park and Williams who obtained a very highly toxigenic race which is now used all over the world.

The bacilli obtained from cases of diphtheria have as a rule a toxicity which can be expressed by stating that from 0·1 ($\frac{1}{10}$) c.c. to 0·02 ($\frac{1}{50}$) c.c. of the filtrate of a ten days' old culture on alkaline broth injected subcutaneously into a guinea-pig of 250 grammes weight kills the animal within five days; rarely 0·01 ($\frac{1}{100}$) c.c. produces this result. By employing the American bacillus under the same conditions toxins can frequently be obtained which give the above-mentioned effect in doses of from 0·005 to 0·003 c.c. and occasionally 0·002 c.c. or even smaller doses (Madsen, and personal experience).

Conditions which modify the Virulence and the Toxigenic Power of the Diphtheria Bacillus.

Various methods have been employed to obtain an elevation of the virulence and of the toxigenicity of the diphtheria bacillus.

(a) *Through association with other germs.*

Roux and Yersin (1890) and Funck (1894) observed that when the diphtheria bacillus and the streptococcus were both found in the throat there was a tendency for the type of infection to be severe. On this observation and certain experiments the hypothesis was based that the bacterial association led to an increase in the virulence of the bacillus. By inoculating rabbits with diphtheria bacilli of low virulence together with highly virulent streptococci they obtained bacilli which retained the newly acquired property on re-inoculation in pure culture. The influence of association on these two micro-organisms has been much investigated, the subject having been approached from various points of view.

Von Dungern (1896–7) did not find that the association of these germs in cultures gave rise to more vigorous growth, whereas Bernheim (1897) and Hilbert (1898) both held that the growth tended to be more rapid and abundant. Hilbert also found that association with the streptococcus gave rise to a more rapid and increased production of alkali and toxin. In many cases where cultures of the diphtheria bacillus were remaining at a standstill, in the atoxic acid phase, the addition of streptococci led to alkali and toxin formation. Hilbert discusses the cause of this increase of toxicity. He points out that rapid growth does not necessarily indicate rapid toxin formation, for Blumenthal (1897) showed that in media to which glucose had been added, vigorous and rapid growth of the diphtheria bacillus took place, while at the same time toxin formation was completely inhibited. Escherich (1894) attributed the rise in virulence obtained in the experiments of Roux and Yersin to passage through the animal body. Hilbert, though believing that the virulence is increased by association, ascribes a considerable part of the increased toxicity to an alteration in the medium, produced by the growth of the streptococci. A filtrate from a streptococcal culture proved an excellent medium for the growth and toxin production of the diphtheria bacillus. Gibier (1897) suggested the association of the streptococcus with the diphtheria bacillus as a practical method of obtaining a powerful diphtheria toxin. He found that if the streptococcus were allowed to grow 12 or 18 hours in a broth, before introducing the diphtheria bacillus, the resulting medium was favourable to the growth and toxigenicity of the diphtheria bacillus.

(*b*) *By passage through animals.*

Bardach (1895) used passage through dogs to elevate the virulence. Trumpp (1896) injected avirulent bacilli together with toxin and found that the bacillus recovered from the dead animal was sufficiently virulent to kill a guinea-pig. Hewlett and Knight (1897) believed that they succeeded in converting Hofmann's bacillus into the diphtheria bacillus by heating the cultures and also by passage through guinea-pigs. Salter (1899) claimed to have obtained the same result by passage through small birds. Louis Martin (1898) cultivated the bacillus within the peritoneal cavity of rabbits in the collodion sacs, which were first employed by Metchnikoff, Roux and Salimbeni in their study of cholera. By the passage of the bacillus in this manner through several rabbits a marked increase in toxigenic power was acquired, e.g., after six passages a bacillus, which formerly gave a toxin killing guinea-pigs at $\frac{1}{10}$ c.c. now gave a toxin killing at $\frac{1}{50}$ c.c. By the same treatment he was able to augment the toxigenic power of the American bacillus though the passage had not increased the virulence. Many of the previous experimenters made no distinction between toxigenicity and virulence, but Martin makes a clear distinction between the two properties.

An interesting point to note in connection with the toxigenic property of the diphtheria bacillus is the persistence with which certain races maintain this character. Park and Williams' toxigenic bacillus, though it has been cultivated on artificial media for 11 years, appears to have suffered no diminution in its toxigenicity.

The Influence of the Medium on the Production of Toxin.

For obtaining powerful toxins the character of the medium is most important. Roux and Yersin (1889–1900) employed simple broth and obtained only weak toxins. Later it was found that the addition of peptone to the broth gave better results. The influence of the amount of peptone was studied by Park and Williams (1896).

They found that as a rule the addition of 2 % and 4 % of peptone gave more powerful toxins than the addition of 1 %. The writer failed to find that 4 % peptone broth gave more powerful toxins than 2 %. The quality of the peptone is probably most important; certain preparations appear to give more powerful toxins than do others, under apparently similar conditions. Witte's peptone is the one in common use.

The reaction of the broth is of great importance.

The growth of the diphtheria bacillus in ordinary broth gives rise to various changes in the reaction. At first the broth becomes slightly alkaline, then there is an acid phase occurring in three or four days. The acid phase may persist or the reaction, after a few days, may become alkaline.

The relation of this acid phase to the presence of glucose in the beef was investigated by Spronck (1895), van Turenhout (1895), Park and Williams (1896), Cobbett (1897), and Blumenthal (1897). They found that where glucose was present except in very small quantities the acid phase was very marked and tended to be permanent. The persistence of the acid phase is deleterious to the growth of the bacillus and to the production of toxin. Madsen (1897) found that the formation of toxin and the degree of alkalinity run parallel, but that while a high degree of alkalinity is usually associated with a high toxicity this is not invariable. Cultures which have an acid reaction when filtered, say after ten days' growth, are atoxic. These observations show the importance of avoiding a marked or persistent acid phase, and many suggestions have been made in this direction. Spronck and van Turenhout (1895), attributing the excessive production of acid to the presence of glucose, recommended that beef which has been kept several days, during which decomposition of the glucose takes place, should be employed. They also suggested the addition of calcium carbonate to the medium, so that when the acid is formed it should enter into combination with the alkaline earth and in this way the medium would remain alkaline, thus aiding the formation of toxin. Nicolle (1896) in opposition to Spronck recommends that the meat should be fresh. Theobald Smith (1899) finds that dextrose is not in itself inimical to toxin production, but that a certain quantity is in fact essential to an abundant accumulation of toxin. He gets rid of the muscle sugar from the beef infusion by inoculating this with a culture of *B. coli* and incubating overnight, but afterwards adds to his medium a measured quantity, about 0·1 % of dextrose. In this way he states that he obtains a greater uniformity in the toxicity of the filtrates.

Park and Williams find that if the medium is made sufficiently alkaline the amount of glucose usually present even in the fresh (two days old) unfermented meat is not sufficiently great to interfere with toxin production. After bringing the medium to the neutral point of litmus they add from 5 to 10 c.c. of normal caustic soda per litre. The writer's personal experience coincides with this.

Martin's medium has been used in the attempt to obtain a medium of more constant composition which would give more uniformly filtrates of a high degree of toxicity. A peptone solution is obtained as follows: the mucous membrane and muscle of five pigs' stomachs are minced and mixed with water acidulated with hydrochloric acid. The proportions are as follows:

Pigs' stomachs, minced	200	grammes
Hydrochloric acid, pure	10	c.c.
Water	1000	c.c.

Digestion is allowed to proceed at 50° C. and is normally completed in 12 hours, but no harm is done by allowing the digestion to go on for 24 hours as no organisms develop in the acid mixture. The mixture is heated to 100° C. which destroys the remaining pepsin, and is then passed through a sieve, or, better, filtered through a thin and loosely packed layer of absorbent cotton wool. The medium is then heated and made alkaline while at 80° C. It is then filtered through paper to remove the precipitate, heated to 120° C., filtered and put in culture flasks in which it is sterilised by heating for half an hour to 115 °C.

This medium by itself is suitable for cultivating organisms, but better results are obtained by mixing it with broth. The peptone prepared in this way gives more constant results than the very variable peptones of commerce.

The broth is derived from veal. After testing the value of fermenting with a yeast to get rid of the glucose Martin came to the conclusion that the best results were obtained by allowing the minced veal to ferment without using any special organism for the purpose. Five hundred grammes of minced veal are mixed with 1,000 c.c. of water and put in the incubator for 20 hours at 35° C., ·5 $^0/_0$ of sodium chloride is added, the fluid made alkaline, and then sterilised. Equal parts of this and the peptone solution are now heated to 70° C., to coagulate the albuminous substances, filtered through paper, made alkaline and sterilised. The sterilisation which gives the best result according to Martin is filtration through a Chamberland filter, but good results are obtained with sterilisation at 120° C. Slight variations under certain conditions are employed. Toxin is obtained by this method which kills a guinea-pig of 500 grammes weight in a dose of $\frac{1}{50}$ c.c.

Martin's medium gives excellent toxins, but in the writer's experience a uniform high toxicity is not attained by this nor by any other process. The use of a boiled and filtered extract of bakers' yeast with the addition of 2 $^0/_0$ peptone as was recommended by Spronck (1898) gave disappointing practical results.

The writer's best results, confirmed by Madsen and Dreyer, have been obtained by the employment of the following method, which is somewhat similar to that described by Park and Williams, and is less prolonged and complicated than that of Martin.

The beef which is employed is that sold by English butchers under the name of "silverside," consisting of part of the muscles of the gluteal region. The meat is used either in a perfectly fresh condition or after it has been kept about seven to twelve days in an ice-cooled air chamber at about 8° C. It has never been frozen nor does it come in contact with the ice. This beef is freed from all fat and fascia and is then finely minced in a mincing machine. Usually 10 to 20 litres of the broth are made at one time. The proportion of beef and water is about one pound (about half a kilo) of beef to 1 litre of a tap water which is fairly alkaline from the presence of lime salts. Appended will be found an analysis of this water.

Chemical Examination.

Determinations	Results in	
	Grains per gallon	Parts per 100,000
Chlorine	1·3	1·8
Equivalent to chlorides (60 $^{0}/_{0}$ Cl) ...	2·1	3·0
Nitric nitrogen	0·28	0·4
Equivalent to nitrates (17 $^{0}/_{0}$ N) ...	1·68	2·4
Nitrites	Absent	Absent
Hardness: permanent ,, temporary	5°	—
Lead, copper, zinc, iron	Absent	Absent
Free ammonia	0·0040	0·0057
Organic ammonia	0·00028	0·0004

The beef and water are then put into a large enamelled ordinary cooking pot with a loose cover and the mixture is kept boiling quietly for 1½ to 2 hours. It is then filtered through Swedish filter paper, the juice at the same time being very thoroughly squeezed out from the beef. A potato or vegetable squeezer is very convenient for this purpose. To the filtrate so obtained are added 2 $^{0}/_{0}$ Witte's peptone and 0·5 $^{0}/_{0}$ sodium chloride. The liquid is then steamed at 100° C. for one hour, and the resulting precipitate is filtered off. At this stage, and while still hot, the filtrate is made neutral to litmus paper, and then 7 c.c. of normal caustic soda are added per litre. It is again steamed for one hour and again filtered. The broth is then put in the culture flasks, usually Erlenmeyer's, and sterilised by steaming for 20 minutes, the temperature being then allowed to run up to 120° C., at which point the gas burner is turned off.

A number of experiments performed on the influence of temperature in the preparation and sterilisation of this broth showed that the temperature employed had little or no influence on the end result.

The toxicity for guinea-pigs of 250–280 grammes weight is shown in the Tables A and B which give the results on two continuous series of toxins obtained by the use of this broth. These include all the toxins prepared and tested during that period and are not selected.

TABLE A.

Diphtheria Toxins—September, 1899, *to June*, 1902.

Minimal lethal dose and time of death in days.

Toxin in c.cs.	Days:—1	2	3	4	5	6	6+
1/500	—	—	—	—	2	—	—
1/400	—	3	8	2	—	1	—
1/300	—	2	8	3	3	1	2*
1/200	—	3	5	4	3	2	5*
1/100	—	10	6	3	—	2	14*
1/50	—	1	—	—	—	—	—
1/30	—	—	—	—	—	—	1

* Not tested in larger doses.

TABLE A—(*continued*).

Total number of toxins, 94.

2 killed within 6 days at 1/500, i.e. 2 %
14 ,, ,, ,, 1/400 ,, 15 %
17 ,, ,, ,, 1/300 ,, 18 %
17 ,, ,, ,, 1/200 ,, 18 %
21 ,, ,, ,, 1/100 ,, 22 %
16 did not kill at 1/100 ,, 17 %

TABLE B.

Diphtheria Toxins from January, 1904, *to June,* 1905.

Minimal lethal dose and time of death in days.

Toxin in c.cs.	1	2	3	4	5	6	6+
1/700	—	—	—	—	—	1	—
1/600	—	—	—	1	—	—	—
1/500	—	1	5	—	1	1	—
1/400	—	2	2	2	1	2	—
1/300	—	1	5	—	1	—	—
1/200	—	1	2	1	3	—	5*
1/100	—	1	—	—	—	—	—

* Not tested in larger doses.

Total number of toxins, 39.

1 killed within 6 days at 1/700, i.e. 2·6 %
1 ,, ,, ,, 1/600 ,, 2·6 %
8 ,, ,, ,, 1/500 ,, 20·5 %
9 ,, ,, ,, 1/400 ,, 23·1 %
7 ,, ,, ,, 1/300 ,, 18·0 %
7 ,, ,, ,, 1/200 ,, 18·0 %
1 ,, ,, ,, 1/100 ,, 2·6 %
5 did not kill at 1/200 ,, 13·0 %

Besides the race of bacillus and the medium employed there are other important factors which must be considered in connection with the preparation of diphtheria toxin.

(c) *Influence of temperature, oxygen and light on the diphtheria bacillus.*

The supply of oxygen.—To obtain abundant growth and good toxin formation a free supply of oxygen is required.

Roux and Martin (1894) suggested the use of Fernbach's flat flasks over which a current of air could be passed by the use of a water pump. Madsen (1896) found on comparing the toxins which were obtained by growing the bacillus in Erlenmeyer's flasks and in Fernbach's flasks with an air current, that the latter method gave weaker toxin, an experience that coincides with the writer's. This is probably due to excessive oxidation of the toxin and to a disturbance of the membrane on the surface of the broth. Though artificial aeration is unnecessary and the form of the culture flask immaterial, it is essential (as was pointed out by Aronson (1894)) that the medium should form a thin layer so that a large surface may be exposed. The amount of material used for inoculation has been suggested as a possible cause in the variation of the toxicity of the different flasks of one series. The best results have been obtained by the writer by inoculating from one broth flask to another. A flask with a good membrane is selected, and a 2 millegramme loopful of membrane is gently floated onto the surface of the medium to be inoculated.

It is now generally agreed that the optimum temperature for the growth of the diphtheria bacillus and for toxin production is 35·5° C., a little below body temperature.

Light must be rigidly excluded from the incubator, or *chambre-étuve*, as it has a deleterious action on the growth of the bacillus.

Period of Maximum Toxicity.

The views regarding the time in which the maximum amount of toxin is produced in a culture have greatly changed. Roux and Yersin (1890–1894) stated that in cultures grown without aeration a strong toxin was not produced under three weeks' incubation, but that when a constant air current was employed two weeks' incubation was necessary. Aronson, however, showed that by inoculating from the pellicle of a highly virulent culture he could obtain a potent toxin in eight days. It is now generally held that the maximum toxicity is reached about the tenth day (Aronson, 1895; Park and Williams, 1896), the time varying between the 5th and the 12th day. Recently Murillo (1903) has pointed out that a secondary rise of toxicity occurs about the 24th day, which he holds is probably due to a liberation of an intrabacillary toxin.

The above tables are formed from rough estimations of the minimal lethal dose, usually expressed by the letters M. L. D. When the M. L. D. is being ascertained for the purpose of determining the constitution of a toxin the doses are more finely graded. For example, if, by a rough test, a toxin has been found to kill a 250 gramme guinea-pig in a dose of about $\frac{1}{300}$ c.c., it is desirable to grade the series of test doses as follows:—0·0028, 0·0029, 0·003, 0·0031, 0·0032, 0·0033, and 0·0034. The smallest dose killing on the fourth or fifth day is regarded by Ehrlich as the M. L. D. The selection of this time-limit is quite arbitrary.

Arrhenius and Madsen (1904) have introduced two new methods by the use of which they assert that it is possible to form a more correct estimation of the M. L. D. than by the use of direct valuation only. One is based on the loss of weight, and the other on the time which elapses between the injection of toxin and the death of the animal. By utilising these two methods many experimental data which would otherwise be useless, become valuable for calculation[1].

Loss of Weight.

There is usually a progressive loss of weight during the first few days after the injection, which reaches its maximum on the fifth or sixth day. From a very large number of experiments with fresh toxins the average loss of weight per day has been calculated. If the maximum loss on the fifth day is represented by 100 the average loss at the end of each day is shown in the following table.

1.	2.	3.	4.	5.	6.	7.	8.	10.	14.	days.
40	52	70	87	100	98	94	78	51	11	average loss of weight.

Having ascertained the loss of weight on any of the first four days after injection of the toxin, it is possible by means of these ratios to obtain a calculated value for the maximal loss of weight on the fifth day. A definite relation is held to exist between such maxima and the dose of toxin.

Calculated maximal loss of weight in grammes:—														
120	100	80	65	60	55	50	45	40	35	30	20	10	0	<0
Dose:—														
1·30	1·20	1·10	1·00	0·92	0·85	0·78	0·70	0·61	0·55	0·50	0·45	0·40	0·35	0·35

Time of Death.

In a similar manner from a large number of experiments they find that if the animal dies in

1 day the dose corresponds to 1·6 minimal lethal doses.
1·5 days „ „ „ 1·4 „ „ „
2 „ „ „ „ 1·25 „ „ „
2·5 „ „ „ „ 1·15 „ „ „
3 „ „ „ „ 1·05 „ „ „
3·3 „ „ „ „ 1·0 „ „ „
3·5 „ „ „ „ 0·97 „ „ „
4 „ „ „ „ 0·91 „ „ „
4·5 „ „ „ „ 0·85 „ „ „
5 „ „ „ „ 0·80 „ „ „

---

[1] Arrhenius and Madsen find that the data obtained by the two methods approximate very closely. Craw and Dean (1907) have recently examined a large number of data from this standpoint. Their results do not altogether support the deductions of Arrhenius and Madsen.

6 days the dose corresponds to 0·71 minimal lethal doses.
7 ,, ,, ,, ,, 0·64 ,, ,, ,,
8 ,, ,, ,, ,, 0·59 ,, ,, ,,
9 ,, ,, ,, ,, 0·55 ,, ,, ,,
10 ,, ,, ,, ,, 0·51 ,, ,, ,,
12 ,, ,, ,, ,, 0·45 ,, ,, ,,
14 ,, ,, ,, ,, 0·4 ,, ,, ,,
>14 ,, ,, ,, ,, $<\genfrac{}{}{0pt}{}{0\cdot4}{0\cdot4}$,, ,, ,,

The Relative Susceptibility of different Species of Animals to Diphtheria Toxin.

The relative susceptibility of animals to toxins is usually recorded in terms of the quantity of toxin required to kill a gramme weight. The guinea-pig, being the animal most frequently employed for experimental work on diphtheria toxin, is usually taken as the standard. The guinea-pig is as a rule wonderfully uniform in its resistance but Ehrlich and Theobald Smith have recorded racial variations in susceptibility, and the English guinea-pig is stated to be more resistant than the German. Ehrlich has found the susceptibility of the pigeon and the guinea-pig to be so nearly equal that it has been possible to use pigeons for test animals, when there has been a dearth of guinea-pigs. Behring (1899) found the rabbit twice as sensitive as the guinea-pig; other observers put them as nearly equal. The rabbit is much less susceptible than the guinea-pig to infection with the living bacillus. The horse requires only a fraction of the guinea-pig's lethal dose per gramme to kill him, and the goat has a similar high susceptibility. The more resistant rat, dog, cow and sheep require several multiples of the guinea-pig's lethal dose. White mice are very resistant requiring as much as 1,000 times the guinea-pig's lethal dose.

Calabrese and Zagari (1895) stated that cold-blooded animals were insusceptible, but Courmont, Doyon and Paviot (1895) found that frogs injected with diphtheria toxin, and kept at high temperatures such as 30° C., lost flesh and became paralysed. Reference has already been made to the susceptibility of the alligator (p. 452).

On the Origin and Nature of the Toxin.

On account of its enzyme-like qualities, Loeffler looked upon diphtheria toxin as a secretion. Gamaleia (1892; quoted by Kossel) thought that the toxin originated by the solution of the toxic bodies of the bacilli, i.e., by a form of autolysis or lixiviation. In favour of this view he adduced the fact that, notwithstanding great bacterial growth, practically no toxin could be found in the culture fluid separated from the bacilli during the first few days of their growth. Guinochet (1892) and Ouschinsky (1893), who attempted to solve the question of the origin of toxin by growing the bacilli in proteid-free nutrient media, came to the conclusion that the toxin was produced by a synthetic process within the bacterial cell. As, however, their results in regard to diphtheria were not confirmed by Fränkel (1894), Hugonnency and Doyon (1896) or Doyon (1896), little or no growth being obtained on their media, much weight cannot be attached to their deductions. Kossel (1896) demonstrated that even a one day old culture contained toxin in the broth. By means of a 1 % solution of caustic soda he succeeded in extracting toxic substances from the bodies of the bacilli killed by chloroform. From a great quantity of bacilli obtained by three days' growth in ten flat flasks, he extracted 10 c.c. of a clear, light yellow fluid which gave no reaction with Millon's reagent, and only a trace of Biuret reaction. This fluid in doses of 0·4 c.c. killed guinea-pigs in less than 48 hours with all the signs of typical diphtheria intoxication.

In consideration of these facts, namely, the rapid appearance of toxin in the nutrient medium, and the very small quantity of toxin obtainable by solution of the bacilli, Kossel concludes that the production of toxin cannot be attributed to a mere autolysis of the toxic bodies of the bacilli. He assumes, therefore, that the toxin is elaborated from the nutrient substances by a metabolic process within the body of the bacillus and is excreted.

Brieger and Boer (1896) found that after the removal of the last traces of soluble toxin from the bacillary bodies (which was best effected by washing with a concentrated ammonium chloride solution), the bodies of the bacilli still contained a highly toxic substance which was characterised by its power of causing necrosis, by its thermostability, and by the fact that its action was not neutralised by antitoxin. These results have frequently been confirmed.

Vicquerat (1902), in a paper with somewhat fanciful views, holds that there are four phases in the diphtheria culture:—

1. Formation of lactic acid by the fermentation of the carbohydrates,
2. Ammonia and para-lactic acid formation from peptone,
3. Solution of the diphtheria bacilli,
4. Toxin formation.

Murillo (1903) from his observation of the secondary rise in the curve of toxin formation (see p. 469) believes in the existence of an intrabacillary toxin without having definite views as to whether maceration, autolysis, or lixiviation is the factor at work in setting it free.

Rist (1903) recovered the bacilli from the filter papers used in filtering cultures during the preparation of diphtheria toxin. The bacilli were heated for 24 hours with a mixture of alcohol and ether, dried *in vacuo* over sulphuric acid, and kept *in vacuo* in the dark. He found, in accordance with Brieger and Boer's results, that antitoxin did not preserve the life of the animal against bacilli treated in this way and made into an emulsion with salt solution. He is inclined to attribute the paralysis, myocarditis, and late death generally, to the action of this intrabacillary toxin.

Notwithstanding these views the writer believes that the trend of opinion amongst experienced workers is to regard toxin formation as due not to a degeneration or death of the bacilli but to a genuine process of secretion.

THE CONSTITUTION OF DIPHTHERIA TOXIN.

The greater part of our knowledge of the structure of diphtheria toxin is due to Ehrlich. Realising that the ordinary filtrate from a diphtheria culture was a very complex body, and that there seemed little probability of obtaining a toxin in a pure state by chemical means, Ehrlich proceeded to investigate the subject from a biological standpoint. He examined for this purpose twelve crude toxins, making for each certain quantitative determinations. The absolute toxicity was accurately measured by ascertaining the exact dose required to kill a guinea-pig weighing 250 grammes on the fourth or fifth day. This is called the minimal lethal dose (M. L. D.) and may be regarded as the unit of Toxin (T). The neutralisation of the toxin by means of an arbitrary quantity or unit of serum was next tested and two limits were fixed which were of great importance in determining the constitution of the toxin[1].

The quantity of toxin which is neutralised by one antitoxic unit is designated by Ehrlich "L_0" (limes nul = Glattwert). The mixture of L_0 and one unit of antitoxin is completely without effect on animals. L_+ (limes Tod = death limit) is the smallest quantity of toxin which, when mixed with one antitoxic unit, causes the death of the guinea-pig within five days. Now if diphtheria toxin were a simple chemical body, $L_+ - L_0 = (D)$ the difference should be, when expressed in lethal doses, equal to one lethal dose. That is, the addition to the L_0 of one M. L. D. or T, should give the L_+ which causes the death of the guinea-pig. To illustrate these points one may take a theoretically pure toxin of which the M. L. D. or T is 0·01 c.c., and the L_0 1 c.c. Then one would find that as T = 0·01 c.c.

---

[1] The antitoxic unit, = "A. U." (I. E. = Immunitätseinheit, Ehrlich) will be dealt with in a later section, but may be taken here as the quantity of antitoxin which is contained in 1 c.c. of a serum of which 0·1 c.c. neutralises the tenfold M. L. D. for a guinea-pig. An A. U. is then capable of neutralising 100 M. L. D.

$$\begin{array}{ll} L_+ = 1{\cdot}01 & = 101 \text{ T.} \\ L_0 = 1{\cdot}00 & = 100 \text{ T.} \\ \hline \text{D (difference)} & = 0{\cdot}01 = 1 \text{ T.} \end{array}$$

Such a state of affairs is, however, never found. To convert the mixture of L_0 and 1 A.U. into the L_+ one must always add more than 1 T. Ehrlich ascertained by working out these constants in the 12 toxins specially investigated that the difference between the L_+ and the L_0 was never a single lethal dose; it varied from 1·7 to 28 minimal lethal doses. Now of these 28 M. L. D. only one is required to kill the animal, the others appear to have been fixed by the antitoxin in such a manner that they produce no effect.

From this it is clear that the absolute toxicity of a toxin and its power of neutralising antitoxin do not stand in direct relation to one another. There must therefore be other non-lethal substances in the crude toxin which have the power of entering into combination with or neutralising antitoxin. On the assumption that a fixed quantity of toxin will always enter into combination with the same quantity of antitoxin, and having regard to the specific relation of the antitoxin to the toxin, the natural deduction is that these non-lethal antitoxin neutralising substances are derivatives or modifications of the toxin, and with this idea Ehrlich calls them "toxoids." One of the strongest proofs of the existence of toxoids is obtained from observing the changes which occur on keeping diphtheria toxins. It is frequently found that while the quantity of toxin neutralised by one unit remains the same there takes place a progressive diminution in the absolute toxicity of the poison. Ehrlich, for example, found that the L_0 value of a toxin was 0·31, and that at first its M. L. D. for guinea-pigs was 0·003. Nine months later the M. L. D. was 0·009, that is, it had only one-third of its original toxicity, whereas its combining power with antitoxin remained unchanged, the L_0 still being 0·31. In this case although the combining power of the toxin had remained unaltered two-thirds of the toxin had become converted into toxoids.

The toxoids, as a rule, are present only in small quantities in fresh cultures; it is only in old cultures or filtrates which have been kept a long time, or which have been subjected to the action of chemical agents, that they are present in large quantity. The toxin molecule may be regarded as possessing a stable, combining or "haptophorous group" (ἅπτω = I grasp) and an unstable "toxophorous group." The bearing of this on Ehrlich's theory of antitoxin production is important and will be dealt with later.

If the toxoids are responsible for the large number of lethal doses in D, it must be due to their having different affinities to antitoxin. Looked at from this point of view of affinity to antitoxin, three groups are conceivable:—

(1) Protoxoids, which possess a greater affinity than the toxin,
(2) Syntoxoids, which have an affinity equal to the toxin, and
(3) Epitoxoids, or toxones, which have an affinity less than the toxin[1].

Ehrlich assumes that the toxin and antitoxin in their combination follow the law of equivalents, and that a toxin unit which has been converted into a toxoid unit is still capable of combining with the same amount of antitoxin.

If protoxoids are present together with toxin in a filtrate and antitoxin is gradually added, the protoxoids, owing to their great avidity for antitoxin, must be saturated before any of the antitoxin will enter into combination with the toxin. Ehrlich uses as a rough chemical analogy the phenomena which occur on the addition of an alkali, analogous to antitoxin, to two acids of different combining affinities, for example, hydrochloric acid, analogue of the protoxoid, and acetic acid, analogue of the toxin. If, for instance, a diphtheria filtrate contains 99 parts of protoxoid and one part of toxin and is completely neutralised by antitoxin, the state of affairs may be represented by the following equation:—

$$\overbrace{\text{99 protoxoid-antitoxin}} + \overbrace{\text{1 toxin-antitoxin}} = \text{physiologically neutral.}$$
$$\text{99 NaCl} + \text{1 acetate of soda} = \text{chemically neutral.}$$

If one adds to the mixture one equivalent of protoxoid, or, in analogous fashion, one equivalent of hydrochloric acid, the re-arrangement resulting may be represented thus:—

$$\text{100 protoxoid-antitoxin} + \text{1 free toxin} = L_+$$
$$\text{100 NaCl} + \text{acetic acid} = \text{free acetic acid;}$$

---

[1] Epitoxoids and toxones. At first Ehrlich believed that the substance with least affinity to antitoxin was like the two others, an altered toxin, and therefore named it epitoxoid, but later the results obtained by the examination of three fresh toxins forced him to the conclusion that it was a primary secretion of the bacillus, and to mark this change of view he adopted the name of toxone in place of epitoxoid.

the elements with the greater affinity combining and leaving those with less affinity uncombined.

If one adds to an L_0 mixture of toxin and syntoxoid further quantities of the original mixture of toxin and syntoxoid, the union of the latter is not influenced by the addition of toxin, and one finds that death results when the added quantity contains a toxic unit. The presence of protoxoids or syntoxoids then does not raise the value of D, since the addition of one toxin equivalent is at once evident.

The influence of the presence of epitoxoids, or toxones, is very different. If one adds toxin equivalents to a mixture containing toxones, the former all seem to disappear without immediate effect, and the toxic action is only shown after sufficient toxin equivalents have been added to use up all the antitoxin previously occupied by the toxone. This is seen in the scheme:—

90 toxin-antitoxin + 10 epitoxoid-antitoxin = physiologically neutral.

If one adds to this 11 toxin units one obtains

100 toxin-antitoxin + 1 toxin free + 10 epitoxoid free = L_+.

From a consideration of these facts, the reason why the difference, D, between the L_0 and the L_+ is not a single lethal dose becomes evident. It is the toxone alone which is responsible for this elevation of the value of D.

The characteristics of the epitoxoids, or toxones, may be ascertained from a study of the effects produced on guinea-pigs by the mixtures of antitoxin and toxin lying between the L_0 and the L_+ doses, namely doses in which the toxin is not completely neutralised, and is yet not present in sufficient quantity to cause the death of the animal on the fourth or fifth day. The effects in this zone must be due chiefly to toxone action since it is here that the toxones, displaced by the toxin from their union with the antitoxin, become free.

Non-lethal doses of toxin, containing from half to three-fourths of a lethal dose, when injected subcutaneously into guinea-pigs produce very constant results characterised by the occurrence of extensive infiltration, at first soft but afterwards becoming indurated, followed by necrosis of the skin and alopecia: paralysis with toxin alone is rare.

Now in the guinea-pigs receiving toxin-antitoxin mixtures between the L_0 and the L_+ doses the infiltration is, as a rule, softer and tends to disappear without necrosis and alopecia. Even when the infiltration has been slight and has rapidly disappeared paralysis is a frequent occurrence, the onset taking place usually about the third week. So common indeed is paralysis under these conditions that Ehrlich regards the toxone as the element in the crude toxin which is alone responsible for the paralysis.

The progressive loss of toxicity in a number of toxins showed an interesting arithmetical relation. Ehrlich points out that a number of diphtheria toxins are so constituted that one antitoxic unit completely neutralises about 100 toxic units, that is, the L_0 dose corresponds to about 100 toxic units. On the other hand, in the majority the L_0 dose contains a smaller number than this, and the number, allowing for experimental error, has the relation to 100 of ½ or ⅓.

Thus Ehrlich found the following numbers

47·5, 54·4, mean 50·9.
33, 32, 33·2, 33·4, 35·7, mean 33·4.

From these experiments it may be argued that:—

(1) Toxins lose their toxic powers, in simple proportions,
(2) The power of fixing the antitoxin is constant,
(3) In many cases one antitoxic unit combines with a quantity of toxin representing 100 toxic units.

These facts compel us to assume that the L_0 of these toxins originally contained 100 T, but that a conversion of the toxin into toxoids occurred in the simple proportion of 1 : 2 or 1 : 3.

In determining the antitoxic value of his standard serum Ehrlich used a toxin which contained equal parts of toxin and toxone. One hundred M. L. D. of that poison therefore contained 100 equivalents of toxin and 100 equivalents of toxone, and the quantity of serum which neutralised 100 lethal doses in reality fixed 200 equivalents of the poison. He concluded that the combining power of the antitoxic unit was 200, and that this unit would always combine with 200 equivalents of all diphtheria toxins whatever proportion of toxin, toxoids and toxones they might contain.

If this neutralising power of one unit is accepted the following formulae can be deduced when

a represents the number of units of toxin contained in L_0.
Z " " " " " toxone " "
and $\beta = D - 1$.

Then $200 - Z$ = the amount of toxin and toxoid,
$200 - a - Z$ = the amount of toxoid,
$L_0 = (200 - Z)$ toxin-toxoid + Z toxone,
$L_0 = (200 - a - Z)$ toxoid + a toxin + Z toxone = 200 equivalents.

Adding 1 unit antitoxin.

1 unit antitoxin + $L_0 = (200 - a - Z)$ toxoid-antitoxin
+ a toxin-antitoxin + Z toxone-antitoxin.

To set one toxone free, since we have 200 − Z equivalents of greater affinity, of which there are a toxin equivalents, it is necessary to add to L_0 $\frac{1}{200 - Z}$ of which $\frac{1}{200 - Z} \cdot a$ is the number of toxin equivalents.

To set Z toxone equivalents free it is necessary to add $\frac{Z}{200 - Z}$ of which $\frac{Z}{200 - Z} \cdot a$ are toxin equivalents, viz., the quantity of toxin solution expressed in toxic units which must be added to 1 antitoxin unit + L_0 to set free all the toxone equivalents which, with the addition of one toxin equivalent, produces the L_+ dose. That quantity may be designated β which is expressed by the difference between L_+ and L_0 minus 1 M.L.D., i.e., D − 1[1], and a may be obtained by dividing the L_0 dose by M.L.D. $a = \frac{L_0}{\text{M.L.D.}}$, so that

$$\beta = \frac{Z}{200 - Z} \cdot a,$$

$$\beta\,(200 - Z) = Za,$$

$$aZ + \beta Z = 200\beta,$$

$$Z = \frac{200\beta}{a + \beta}.$$

This may be more conveniently expressed in the following way[2].

Let Z = no. of toxone equivalents in the L_0 dose,

a = „ toxin equivalents in the L_0 dose, i.e., toxin expressed as M.L.D.

Then each of the (200 − Z) toxin-toxoid equivalents may be considered to contain $\frac{a}{200 - Z}$ toxin equivalents.

Now if one adds an amount of toxin (P) expressed in M.L.D. which will bring the L_0 dose up to the L_+ dose − 1 M.L.D., the 200 antitoxin equivalents will now be able to combine with an amount of toxin = a + P.

$$\therefore \text{ each equivalent now contains } \frac{a + P}{200} \text{ toxin.}$$

But by theory Z of these equivalents must be exactly equal to the amount of toxin added,

$$\therefore Z\,\frac{a + P}{200} = P,$$

$$\text{i.e., } Z = 200\,\frac{P}{a + P}.$$

$$Z = \text{toxone} = 200\,\frac{(L_+ - L_0 - 1)}{L_0 + (L_+ - L_0 - 1)} = \frac{200\,(L_+ - L_0 - 1)}{L_+ - 1}.$$

---

[1] As shown by Park and Atkinson (1898) and Madsen (1899) there is an error in deducting 1. This would be correct only in dealing with a theoretically pure toxin: it should be a fraction of 1.

[2] I am indebted to Dr J. C. G. Ledingham for this solution.

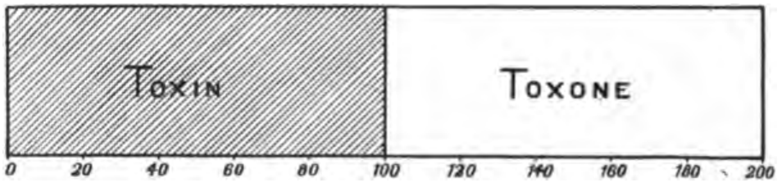
By calculating the number of toxone equivalents present in the L_0 dose of different filtrates one finds the numbers 25, 33, 33, 50, 66, 67, and 100. These numbers show a similar proportional relation to 200 as is observed in connection with the numbers of toxin equivalents.

Park and Atkinson pointed out that the neutralising value of a lethal dose of toxin is lowest when the first considerable amounts of toxin have appeared in the culture. After a short period as the toxin in the culture increases, the neutralising value of the fatal dose begins to increase at first rapidly and then more slowly. After the cessation of toxin production the neutralising value of the fatal dose increases till it may reach five to ten times its original amount. This is quite in accordance with Ehrlich's view of toxoid formation. They point out, however, that the changing of this limit, e.g. to the sixth or seventh day, would entirely alter the values of the L_+ and L_0 and, therefore, the results of his formula. They comment on the difficulty or impossibility of determining the L_0 dose. While refusing to admit the value of Ehrlich's formulae, they admit the great practical value of employing his antitoxic unit as a standard.

The Method of Partial Saturation.

A still more intimate knowledge of the constitution of a diphtheria toxin may be obtained by using the method of partial saturation of the toxin by antitoxin. This method has given most valuable results in the hands of Ehrlich and Madsen, on whose work this account is based. The results obtained depend upon the difference of avidity for antitoxin possessed by the various constituent parts of the toxin. To give a graphic record of these results Ehrlich has introduced a form of schema to which he gives the name of "Toxin Spectrum." Along the axis of the abscissa, which is divided into 200 equal parts representing the 200 equivalents of poison, he distributes the toxin constituents from the left to the right, putting to the left those with the greatest affinity, viz. the protoxoids, and to the right those which possess the least affinity, viz. the toxones. The shaded ordinates indicate the amount of pure toxin present at each particular part of the abscissa, and those unshaded show the amount of toxoid and toxone.

The L_0 of a toxin containing no toxoids but only pure toxin and toxone in equal parts, would be represented by the following figure.

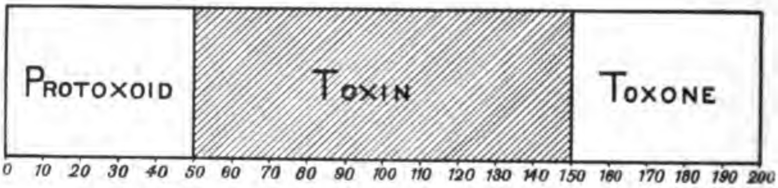


If in this case $\frac{200}{200}$ of an A.U. is added to the L_0 of toxin we have complete neutralisation. If $\frac{199}{200}$ of an A.U. is added, a small quantity of the element with the least affinity to antitoxin will be unneutralised, i.e. a certain amount of toxone. When $\frac{150}{200}$ of an A.U. is added only toxone remains unneutralised.

" $\frac{100}{200}$ " " " " "
" $\frac{99}{200}$ " " 1 lethal dose + the toxone remains unneutralised.
" $\frac{98}{200}$ " " 2 lethal doses + the toxone " "
" $\frac{60}{200}$ " " 40 " " " " "
" $\frac{10}{200}$ " " 90 " " " " "
i.e., the mixture has enough toxin to kill 90 guinea-pigs.
" $\frac{1}{200}$ of an A.U. is added 99 lethal doses + the toxone remains unneutralised,
i.e., the mixture has enough toxin to kill 99 guinea-pigs.

A toxin of such simple structure as represented here has no actual existence.

The L_0 of a toxin containing 50 protoxoid equivalents, 100 toxin equivalents, and 50 toxone equivalents, will be represented thus:—



The $L_0 + \frac{200}{200}$ of an A.U. will not kill a guinea-pig nor produce symptoms.

$L_0 + \frac{199}{200}$ A.U. results in 1 toxone equivalent being free.

$L_0 + \frac{150}{200}$ A.U. results in 50 toxone equivalents being free, and does not kill one guinea-pig.

When a large number of toxone equivalents remain unneutralised we get a transitory oedema at the site of inoculation but not death. Paralysis and late death may follow.

With $L_0 + \frac{149}{200}$ A.U. the 50 toxone equivalents and 1 toxin equivalent are unneutralised and the mixture kills 1 guinea-pig.

Similarly $L_0 + \frac{148}{200}$ A.U. kills 2 guinea-pigs.
$L_0 + \frac{147}{200}$ " 3 "
$L_0 + \frac{100}{200}$ " 50 "
$L_0 + \frac{70}{200}$ " 80 "
$L_0 + \frac{50}{200}$ " 100 "

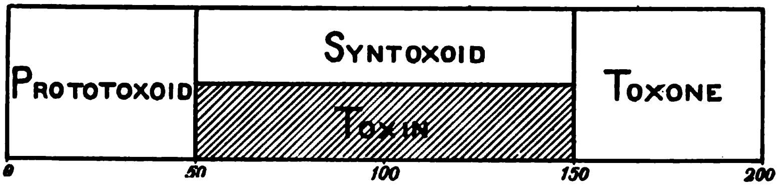
On the addition of lesser quantities than $\frac{50}{100}$ A.U., since all the available toxin is free, no more than the 100 guinea-pigs are killed. On the existence of this non-toxic zone is based the chief argument in favour of the existence of protoxoids.

$L_0 + \frac{50}{200}$ kills 100 guinea-pigs.
$L_0 + \frac{49}{200}$ " "
$L_0 + \frac{1}{200}$ " "

The facts may be stated, perhaps more clearly, in another way.

If we add to the L_0 dose which we know contains x lethal doses, $\frac{1}{200}$, $\frac{2}{200}$, $\frac{3}{200}$, $\frac{4}{200}$, and so on up to $\frac{50}{200}$ of an A. U. we do not find any diminution of the toxicity of these successive mixtures; each still contains x lethal doses. Up to a certain point we seem to have added the antitoxin in vain; it disappears in the mixture without reducing its toxicity. In the example taken this happens until we reach $\frac{51}{200}$ when the number of lethal doses is found to be $x - 1$, $\frac{52}{200} = x - 2$, and so on.

The same toxin may undergo a change, half the toxin being converted into syntoxoid; the dichotomous division or "hemitoxin-stadium" of Ehrlich. The L_0 now contains only 50 toxin equivalents:—

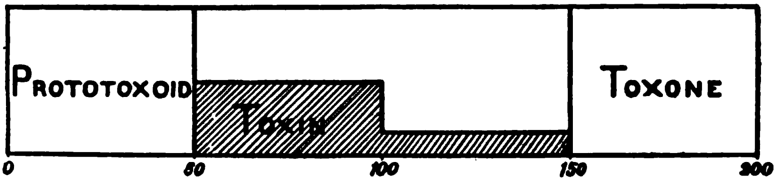


The toxone part of the spectrum will be found unaltered:

$L_0 + \frac{200}{200}$ kills 0 guinea-pigs.
$\frac{150}{200}$ " 0 "
$\frac{149}{200}$ " 0 "
$\frac{148}{200}$ " 1 "
$\frac{147}{200}$ " 1 "
$\frac{146}{200}$ " 2 "
$\frac{145}{200}$ " 2 "

It will be noted that now the mixture containing the L_0 dose and $\frac{149}{200}$ A. U. does not kill a guinea-pig, to do so the mixture must contain $\frac{148}{200}$ A. U., and to kill two guinea-pigs $\frac{146}{200}$ must be added, that is, to kill each additional guinea-pig, instead of diminishing the amount of antitoxin added by one antitoxin equivalent, it is necessary to diminish by two antitoxin equivalents, that is by $\frac{2}{200}$ A. U. instead of by $\frac{1}{200}$ A. U. In other words, half the substance using up the antitoxin is now atoxic.

If we assume a still further weakening of the toxin so that the L_0 dose contains now only 30 toxic units, the distribution may be represented thus:—



Then $L_0 + \frac{200}{200}$ kills 0 guinea-pigs.
$\frac{150}{200}$ „ 0 „
$\frac{141}{200}$ „ 0 „
$\frac{140}{200}$ „ 1 „
$\frac{145}{200}$ „ 1 „
$\frac{130}{200}$ „ 2 „
$\frac{125}{200}$ „ 2 „
$\frac{120}{200}$ „ 3 „
$\frac{100}{200}$ „ 5 „
$\frac{99}{200}$ „ 5 „
$\frac{98}{200}$ „ 6 „
$\frac{97}{200}$ „ 6 „
$\frac{96}{200}$ „ 7 „
$\frac{90}{200}$ „ 10 „
$\frac{50}{200}$ „ 30 „
$\frac{1}{200}$ „ 30 „

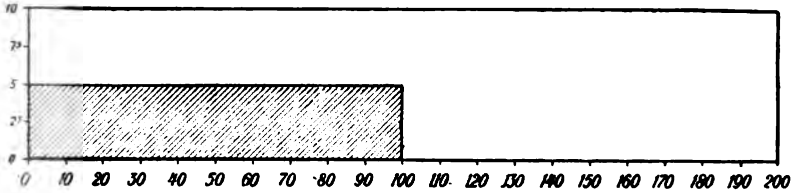
Neither the protoxoid nor the toxone zone is here altered. In the part of the spectrum from 150 to 100, the distribution of toxin and toxoid is such that to liberate one toxin equivalent it is necessary to liberate nine toxoid equivalents.

Ehrlich (1898–1903) has shown how this method may be applied to the study of the different phases through which a toxin passes. A toxin, No. 5, had at first the following constants :

$$L_0 = 0{\cdot}125 \text{ c.cm.}$$
$$L_+ = 0{\cdot}25 \text{ c.cm.}$$
$$\text{M.L.D.} = 0{\cdot}0025 \text{ c.cm.}$$

It will be therefore seen that the L_0 contained 50 M.L.D., and the L_+, being twice the L_0, contained 100 M.L.D.

The toxin had three distinct phases in so far as its toxin content was concerned.



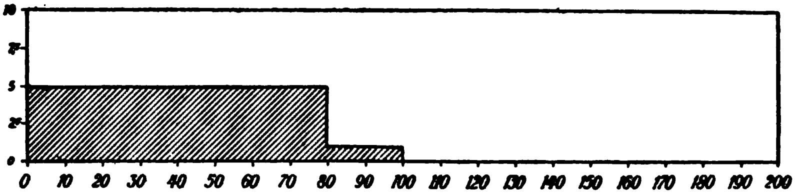
In the First Phase the toxin had the distribution shown in the above figure, that is, it showed a pure hemitoxin. Every antitoxin unit added to the L_0 dose reduced the toxicity by one half M.L.D., that is, by the addition of every $\frac{2}{200}$ A.U. the mixture was lethal to one guinea-pig less. This happened only during the addition of the first hundred $\frac{1}{200}$ A.U., the addition of more antitoxin only influenced the toxone action.

Where the hemitoxin zone of a toxin reaches in the L_0 mixture to 100 what will the L_+ dose be ? The L_+ will be reached when 200 equivalents of hemitoxin are present and when one M.L.D. is left free ; then theoretically we must multiply the

L_0 by $\frac{202}{100}$ to get the L_+ for $\frac{L_+}{L_0} = \frac{202}{100}$. This gives the L_+ as 0·253 and the observed value is 0·25, a remarkably close agreement with the calculated value.

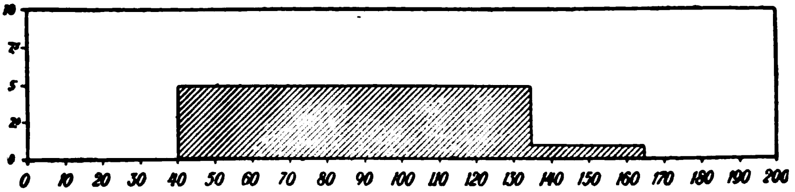
In the Second Phase the L_+ had increased to 0·26 and the M. L. D. had risen from 0·0025 to 0·003. The L_0 dose now contained 42 instead of 50 M. L. D.

The distribution of the toxin is represented in the following figure. From 80 to 100 only $\frac{1}{10}$ of the toxin now remains unaltered. If the fall in toxicity be due to a conversion of the end, or tritotoxin part of the hemitoxin, into toxoid so that only $\frac{1}{10}$ of the toxin is left unchanged, then to obtain the L_+ from the L_0 we must multiply the L_0 not by $\frac{202}{100}$ but by $\frac{210}{100}$. By this calculation we obtain the L_+ as 0·262, again a remarkable agreement with the observed value 0·26 "and a little over."



In the Third Phase there was no change in the toxin region but a loss of toxone had occurred which accounted for a change in the L_0 dose from 0·125 to 0·21.

In the Fourth Phase the L_+ was still ·26 but the M. L. D. had risen from 0·003 to 0·004 so that 22 M. L. D. had disappeared from the L_0.



As shown in the spectrum figure above the disappearance was due to the appearance of an extensive prototoxoid zone affecting the first 40 toxic units of the spectrum. No loss, however, in the combining units had taken place; the toxophorous group alone and not the haptophorous group was affected.

The schemes given will suffice to illustrate the method introduced by Ehrlich to record the constitution of toxins, but a simplicity of structure such as illustrated is never met with in practice. The toxins, and also probably the toxones, do not occur in the form of simple bodies, but fall into various sub-divisions which are differentiated by Ehrlich according to their avidity for antitoxin, so that in a descending scale we have proto-, deutero-, and tritotoxins.

Still further complications exist for we must assume that every one of these varieties of toxin consists of equal parts of two modifications, which have the same relation to antitoxin but differ from one another in their relations to external disturbing influences. The one, the α-modification, is in all toxins readily converted into toxoid. The second, or β-modification, varies in stability in the different groups as follows:

β-modification of deuterotoxin, very stable.
„ prototoxin, less stable.
„ tritotoxin, very unstable.

Although the conversion of the β tritotoxin into toxoid begins early it is never complete; traces of toxin are always present in this part of the spectrum. Later the β-prototoxin disappears altogether. Finally the β-deuterotoxin remains with the trace of β-tritotoxin, and at this stage the changes cease and a stable condition ensues. The possibility of obtaining suitable constant test toxins depends on the stability of the β-deuterotoxin.

To explain certain phenomena Ehrlich had to assume the existence of toxonoids. Madsen and Dreyer (1901) found that a mixture which was completely neutral for guinea-pigs was still acutely toxic for rabbits, and that mixtures containing even less toxin than this L_0 mixture were capable of producing paralysis in rabbits. To obtain, therefore, the L_0 value for rabbits it was necessary to go considerably below the L_0 value for guinea-pigs.

From these results it appeared that toxin-antitoxin mixtures which showed typical toxone or paralysing action in one species, the rabbit, showed no observable action on another species, the guinea-pig, and further that what produced paralysis, or toxone action, in one species (the guinea-pig) produced toxin (acute lethal) action in another species (the rabbit).

Ehrlich (1903) explained these results by the assumption that there were three varieties of toxin, each having a different degree of avidity for antitoxin:—

(1) Toxin, with the greatest avidity, acutely toxic for rabbits and guinea-pigs and more toxic for the former,
(2) toxone, acutely toxic for rabbits, killing guinea-pigs with paralysis,
(3) Toxonoids harmless to guinea-pigs, causing paralysis in rabbits.

A further investigation of the question by Morgenroth (1904) has shown that the assumption of toxonoids, so far as concerns these experiments, is unnecessary. He found that the explanation of the results depends on two factors. Ehrlich, finding that no difference could be observed whether the mixtures were injected 15 minutes after the toxin was added to the antitoxin or after standing together 24 hours, came to the conclusion that the reaction was a rapid one and was completed within 15 minutes. This appears to be quite true only so far as subcutaneous injection into guinea-pigs is concerned. Dreyer and Madsen used in their experiments subcutaneous injection in the case of the guinea-pig and intravenous injection in the case of the rabbit. Now Morgenroth has found that when the toxin-antitoxin mixtures, which show only toxone action when injected subcutaneously into guinea-pigs, give rise to acute toxic action on intracardiac injection. He further found that if a long enough time, say 24 hours, is given for complete union to take place between toxin and antitoxin before the intravenous injection, the mixture, which is completely neutralised for the guinea-pig, is also neutralised for the rabbit.

CONDITIONS WHICH MODIFY TOXIN.

Time.

Toxins are very unstable substances, and a certain period must elapse after filtration before an equilibrium in their constitution is established. Even then their stability is very uncertain, and without any obvious cause they may within a short time undergo great alterations in their composition. The factors at work in producing the changes, which occur with the lapse of time, are very obscure and have eluded investigation. An analogy may be found in the difficulty of determining the exact processes which play a part in the maturing of a wine.

Several of the modifying factors to be discussed are no doubt concerned in the production of the changes.

Light.

Light, especially in the presence of oxygen, has a very deleterious action on toxin. A few days' exposure to diffuse daylight, or a few hours' exposure to direct sunlight, causes a marked deterioration. Piazza (1895) found that the red and violet rays (the heat and actinic rays) of the spectrum, were active, the yellow rays inactive.

Temperature.

Effect of heat on diphtheria toxin.

Exposure to a temperature of 58° C. for two hours, or 100° C. for 20 minutes, almost completely destroys the toxicity of filtrates. Toxin precipitated with calcium phosphate and dried is much more resistant; it withstands a temperature of 70° C., and is not much affected even by a temperature of 100° C. for 20 minutes. Behring holds that toxins heated for half an hour to 100° C. are still capable of producing certain toxic effects on goats and horses.

Effect of low temperatures.

Diphtheria toxin is unaffected by low temperatures. Sudden changes of temperature appear to be injurious.

Action of electricity on toxin.

Smirnow (1894–95) stated that by means of electricity toxin could be modified and converted into substances which acted like antitoxin. His results were obtained by the employment of a continuous current of low tension. Krüger (1895) by the same procedure obtained confirmatory results, while D'Arsonval and Charrin (1896) obtained similar results with alternating currents of high frequency.

For a brief period the hope was entertained that it might be possible to obtain antitoxins without the intervention of the animal organism. Such an idea had soon to be abandoned. Marmier (1896) investigated the subject in a critical and independent spirit, with the object of discovering whether the changes produced in toxin are due to the electricity itself or due to secondary phenomena, the result of electrical action. From his investigation he concluded that continuous or alternating currents of low frequency destroy or attenuate toxins by the production of hypochlorites or chlorine from the sodium chloride present in the toxin. A series of experiments performed by L. Martin, proved that the quantity of hypochlorites present in Smirnow's experiments was greater than was necessary to protect a guinea-pig from a lethal dose of toxin. Currents of high frequency have, apart from the heat evolved, not the slightest attenuating action on toxins.

Oxygen.

Oxygen also destroys toxin. Hence, toxin conserved under toluol retains its toxic properties better than toxin whose surface is in contact with the air. Brieger and Boer (1896) found it to be much more affected by oxidising than by reducing agents.

Peroxides and Oxydases.

Sieber (1901) found that diphtheria and tetanus toxin were inactivated by peroxides and by oxydases of animal and vegetable origin.

Action of Chemical Agents.

Acids. Roux and Yersin found that lactic and tartaric acids almost completely abolished the toxicity of the toxic filtrates. Boric acid, borates and phenol act in the same way, but less vigorously. On neutralising the acidulated toxins some of the original toxicity is recovered. Behring (1894) also found that the inorganic acids acted deleteriously on toxin. Nevertheless he observed that even 1 % hydrochloric acid left a considerable amount of toxin unchanged. More recently Ritchie (1901) has also observed that diphtheria toxin is capable of resisting the action of hydrochloric acid.

Alkalies. While Behring found that a 2 % solution of sodium hydrate had less influence on toxin than 1 % hydrochloric acid, Ritchie (1901) found that diphtheria toxin was very susceptible to its action.

Toxin modified by these means is still capable of producing immunity.

Behring found that toxin was unaffected by 1 % carbolic acid and also by other benzol derivatives and by pure iodoform and chloroform acting in the dark. The metal salts were also harmless, provided they did not act as oxidising agents or yield free chlorine or iodine. Only formaldehyde or iodine-trichloride had a greater effect than the acids. L. Martin observed that the hypochlorites acted injuriously on toxin, and Salkowski (1898) found that antiseptics in strong concentrations exerted a markedly destructive action.

The action of chemical agents on toxin is further dealt with in the section on the purification of toxin (p. 448).

Effect of Bacterial Growth on Toxin.

The growth of living germs, which have contaminated a toxin, exerts a markedly attenuating action. The rapidity and extent of the deterioration depend on the nature of the organisms, and their capacity to attack the different constituents of the broth. On this subject very little is known, but as a rule it is found that the growth of a mould has a much less injurious influence on a toxin than the growth of a bacterium. Nobécourt (1900) found that yeasts caused an attenuation of toxin.

The Purification and Isolation of Diphtheria Toxin.

All attempts at the isolation of diphtheria toxin in the pure state have ended in failure; the negative results, however, are valuable and interesting from the historical standpoint.

The first effort was made in this direction by Loeffler who by the addition of alcohol to a glycerine extract of a broth culture obtained a precipitate, easily soluble in water, which produced the pathological effects of the toxin. Roux and Yersin early observed that precipitates caused by the action of various substances, such as calcium phosphate, on toxic filtrates tended to carry the toxin down with them. Brieger and Fraenkel (1890) were the first to systematically investigate the subject, and as the result of their work concluded that toxin belonged to the serum albumin group and gave it the name of "toxalbumin." They obtained this substance by the use of such precipitating agents as ammonium sulphate, sodium sulphate, and alcohol. Their best results were obtained with alcohol, and their method was briefly as follows:—the broth filtrate, reduced by evaporation to one-third of its volume, was added to a ten-fold volume of absolute alcohol slightly acidulated with acetic acid. After the mixture had stood 12 hours in the ice-box, the precipitate was removed by filtration, re-dissolved in water, and again precipitated. This was repeated six to eight times, till the precipitate when dissolved in water gave a perfectly clear solution. The final precipitate was dialysed and dried *in vacuo* at 40° C. In this way a white powder was obtained which was not precipitated from its watery solution by boiling nor by the action of sodium chloride, sodium or magnesium sulphate, lead acetate or nitric acid, but gave all the other proteid reactions, including precipitation by carbonic acid. It was, therefore, closely allied to the albumoses. Its approximate percentage composition was 45·35 % carbon, 7·13 % hydrogen, 16·33 % nitrogen, 1·39 % sulphur, 29·80 % oxygen.

Wassermann and Proskauer (1891) employed an elaborate modification of this method.

Brieger and Boer (1896) carried out a number of experiments with the object of separating the toxins of diphtheria and tetanus from all other substances, such as the inactive albuminates to which they are bound on precipitation.

They succeeded in obtaining double compounds with zinc salts. The separation of the toxin from these compounds is, however, difficult or impossible on account of the ease with which it undergoes destruction in the presence of certain reagents, such as carbonic acid, sulphuretted hydrogen, sodium phosphate and various acids, which consequently are not available.

On the other hand, alkaline and neutral salts of the alkalis or alkaline earths, and especially certain ammonia salts are not markedly injurious and are thus suitable for the process. The method which gave the best results with diphtheria toxin was carried out as follows. Two volumes of 1 % zinc chloride solution were added to the sterile filtrate. The precipitate was washed and shaken with a volume of ammonium carbonate (3 to 6 %) equal to the original volume of the broth culture. Ammonium phosphate was added until solution of the precipitate took place and the supernatant fluid began to grow turbid owing to the presence of zinc phosphate. The deposit was allowed to settle and was removed by filtration. The filtrate was saturated with ammonium sulphate. The resulting precipitate contained the diphtheria toxin. The accompanying peptone was got rid of by filtration, after the repeated addition of finely powdered sodium sulphate. The toxin thus obtained gave no proteid reaction and was optically inactive.

Wassermann and Proskauer and Brieger and Boer succeeded by their methods in obtaining the toxic substance from the organs of animals that had died of diphtheria intoxication.

A substance of very great physiological activity is not obtained by any of the methods which have been described. During the manipulation a great loss of toxin occurs.

THE EFFECT OF THE TOXIN ON ANIMALS.

(*a*) *Subcutaneous Injection.*

Latent period. When a lethal dose of toxin is injected subcutaneously into a guinea-pig no immediate effect is produced. The occurrence of a latent or incubative period is a very characteristic phenomenon of bacterial toxin action, and it is always observed however large a dose is injected. If many hundred or thousand multiples of the single lethal dose are injected into an animal the incubation period may be curtailed, but it is not abolished. In the case of diphtheria toxin the latent period may be reduced to 8–12 hours.

Local effect. A soft oedematous swelling is observed at the site of inoculation after about 24 hours. If the dose has been large, especially in the case of certain toxins, this oedema may extend from the pubes to the axilla and clavicle. This swelling becomes firmer during the following days and necrosis of the skin may occur. Alopecia of the affected area may also be present. The time at which necrosis occurs varies greatly according to the particular toxin injected. With certain toxins necrosis takes place when the dose is just below the lethal limit. (For morbid appearances of local lesions of lymphatic glands, etc., see p. 268.)

General Effect.

Temperature. In the first 24 hours there may be a slight rise of temperature which, in the case of a lethal dose, is succeeded by a fall which continues until death occurs, when the temperature may be several degrees below normal.

Effect on the body weight. During the first 24 hours, or incubation period, the normal increase in the weight of the animal may be undisturbed, and the animal's weight may even rise 6 to 10 grammes. When a lethal dose has been injected a marked fall in the weight is observed in the second 24 hours, and continues until death occurs. If the animal survives the injection for five or six days it may recover, feed well, gain in weight, and appear to be perfectly healthy. Notwithstanding these favourable appearances paralysis may subsequently develop.

Paralysis. The paralysis may appear from the fifteenth to the thirtieth day, rarely earlier or later. The hind-limbs are usually first attacked though sometimes the first signs are apparent in the fore-limbs.

When the hind-limbs are first attacked the paralysis may ascend and affect the trunk and fore-limbs. In such cases the animal lies on its side unable to move, but if food is brought within its reach it may be able to eat quite freely as the muscles of mastication may not be affected. Recovery in such cases is by no means infrequent. The paralysis may progress, affecting the movements of the thoracic muscles and diaphragm, and result in respiratory death. Death, however, may be due to heart failure associated with degeneration of the vagus.

In certain cases of sudden death in guinea-pigs, which had apparently recovered from the acute intoxication and shown no signs of paralysis, the writer has found extensive fatty degeneration of the cardiac muscle to be the only marked morbid change.

Experimental diphtheria paralysis occurs most frequently when injections of living bacilli or of toxin-antitoxin mixtures have been employed, but it can occur from toxin alone. Ransom (1900), Madsen and Dreyer (1902), and the writer have all observed paralyses following injections of toxin.

(*b*) *Intra-cerebral Injection.*

The effects of intra-cerebral injections of toxin were first described by Roux and Borrel (1898). These authors found that toxin injected into the brain was more rapidly fatal than when injected subcutaneously, and that smaller doses were required. Intra-cerebral injection of toxin causes paralysis within 12 hours, often followed by death, the usual lesions being observed, fluid in pleural cavity, congestion of suprarenal glands, etc.

In animals which have previously received large doses (40 c.c.) of antitoxin a little toxin given intra-cerebrally causes death. In this case the disease is confined to the nervous system, and it lasts longer. At the autopsy none of the usual morbid changes are present. All the organs, excepting those of the nervous system, are protected by the antitoxin.

The writer has found that, as the result of intra-cerebral injection of diphtheria toxin, typical late diphtheria paralysis, beginning even after an interval of weeks during which the animal seems perfectly well, may occur in addition to the acute effects described by Roux and Borrel. This effect has been obtained with pure toxin alone and toxin-antitoxin mixtures. A toxin which, on subcutaneous injection, killed in a dose of $\frac{1}{300}$ c.c., caused typical diphtheria paralysis when injected intra-cerebrally in a dose of $\frac{1}{2000}$ c.c.

Physiological Effects on the Heart and Blood Circulation.

Heart failure is the ultimate result of diphtheria intoxication, but whether this is due to the direct action of the toxin on the heart, or is secondary to a vaso-motor paralysis is still a matter of dispute.

As the pathological changes have been described in a previous section (Chapter VII) only a brief reference to those changes which have a bearing on the physiological effects will be made here. In acute toxic cases cloudy swelling of the heart muscle is present within 24 hours, and in cases where the intoxication has lasted 36 to 48 hours extensive fatty degeneration of the fibres is already present, the fat globules being easily demonstrated by acetic acid, perosmic acid, or Sudan red. Romberg, quoted by Heinz (1905), and Mollard and Regaud (1897–99) have described minutely the changes observed in dogs as the result of both small and large doses of toxin causing death from the third to the fifteenth day. They concluded that the marked changes indicated that the weakening of the myocardium is one of the causes of death, but in no case did they observe the symptoms of a typical acute myocarditis.

Late in diphtheria intoxication, often shortly before death, the blood pressure falls as was shown by Enriquez and Hallion (1895), and Beck and Slapa (1895). Romberg and Pässler (1899) came to the conclusion that the fall in blood pressure which occurs at a certain stage of the disease is not due to insufficiency of the heart but to paralysis of the vaso-motor centre. They arrived at this conclusion from the observation that stimuli acting directly or reflexly on the vaso-motor centre of healthy animals, and thus causing a marked rise in the blood pressure, produce little or no effect on the blood pressure of animals suffering from diphtheria intoxication. On the other hand, they found that the heart was able to deal effectively, as evidenced by a rise in the aortic blood pressure, with an increased volume of blood when this was supplied to it by abdominal massage. The former experiment proved inefficiency of the vaso-motor centre, the latter efficiency of the heart. In regard to the latter observation, however, Beck and Slapa found that when the fall in blood pressure was rapid, going on to death, compression of the abdominal aorta did not cause a rise of pressure, proving the severe injury which the heart had suffered. Enriquez and Hallion in their investigations found that there was progressive paralysis of the vaso-motor centre, for stimulation of the crural nerve or asphyxiation failed to produce a response; the blood vessels themselves were not paralysed, for stimulation of the vaso-constrictors of the vessels was followed by the usual contraction. Pressure on the aorta caused only a comparatively slight rise of blood pressure.

Stejskal (1902) considered that the methods employed by Romberg and Pässler were not satisfactory. A proper estimate of the working capacity of the heart can be ascertained by measuring not only the carotid pressure but also the left auricular pressure, and the ratio between these two gives the quotient of the heart's work.

He used very large doses in dogs and got the effects so rapidly that it is questionable if they were produced by diphtheria toxin solely, or by a mixture of substances such as albumoses from the broth. He concludes that vaso-motor paralysis occurs at an early period but that there are stages when the centre is still capable of stimulation and yet the heart itself fails to respond. The effects of diphtheria toxin on the circulatory system then must be due to both vaso-motor paralysis and injury to the heart itself.

Rolly (1899) thinks that the early fall in blood pressure is due to vaso-motor paralysis, but that this is soon followed by paralysis of the heart, which in spite of artificial respiration rapidly causes death. This heart paralysis is not secondary to the insufficient supply of blood, causing loss of excitability, as was suggested by Romberg and Pässler, but is a direct paralysis. Rolly points out that even when vaso-motor paralysis is complete and the blood pressure is quite low, if artificial respiration is maintained, the heart goes on beating for hours. Rolly also by Hering's method of employing a heart-lung circulation in an animal 24 hours after the injection of diphtheria toxin demonstrated beyond doubt, that in experimental diphtheria intoxication neither paralysis of the vaso-motor centre nor stoppage of respiration can alone be regarded as the cause of death, but that independent of these actions, and even when the heart is separated from the vascular system, heart-death occurs. Rolly could not confirm Fennyvessy's (1896) observation that diphtheria toxin acts directly on the isolated frog's heart, nor could he find evidence of direct toxic action on the rabbit's heart isolated by Hering's method. Even when 60 lethal doses were introduced into the small volume of blood in the heart-lung circulation, the heart continued to beat strongly and regularly for two hours. The toxin never produces an immediate toxic effect on the heart, but after a definite latent period a heart paralysis comes on independent of the earlier vaso-motor paralysis. After the latent period has passed, replacement of the toxic blood by normal blood can never save the life of the animal, even when it is introduced before the onset of the heart symptoms. According to Courmont and Doyon's theory of toxin action the latent period is due to the fact that the injected toxin does not itself give rise to the toxic effects, but acting like a ferment causes the formation of the toxic substance which acts directly on the animal body. Rolly however failed, as many others have failed, to find this substance in the blood, for the blood taken from animals far advanced in diphtheria intoxication produced no effect on healthy animals. The toxic effect on the heart, according to Rolly, is due to an absorption of toxin by the heart muscle causing a functional change. He thinks that the sudden onset of the heart symptoms and their rapid development are against the view that they are due to anatomical changes.

According to Heinz (1905) the diphtheria toxin acts injuriously from the outset, for even at the end of the latent period (20 hours) marked cloudy swelling and even fatty degeneration are visible in the muscle fibres. He points out that in considering the influence of the lesions accompanying diphtheria intoxication on the working capacity of the heart, its extraordinary reserve power must be kept in view. When the pathological changes are continuously progressive a stage must come when the heart begins to flag, the beat becomes weak and arhythmic, and the blood pressure sinks. The sudden onset and rapid progress of the heart failure are not, therefore, as was maintained by Rolly, incompatible with the view that the anatomical cardiac lesions are the cause of the heart failure and fall of blood pressure.

THE FATE OF THE TOXIN IN CORPORE.

The fate of diphtheria toxin *in corpore* depends on various factors; the susceptibility or insusceptibility of the injected animal and the manner of introduction of the toxin being among the most important. The subject will be dealt with under the following headings:

1. In susceptible animals.
2. In refractory animals.
3. Behaviour in the alimentary canal.
4. Behaviour when applied to other mucous membranes.

1. *In Susceptible Animals.*

When toxin is injected into the blood stream of susceptible animals it disappears with remarkable rapidity. Bomstein (1898) investigated this point by injecting rabbits intravenously with toxin and estimating the toxicity of the blood per c.c. at the time of introduction of the toxin and at subsequent intervals of time. The fall in the toxicity is shown in the following short table:

	Hours after injection :—	1	3	5	8	10 to 12
Fraction of original toxin injected	When original toxicity per c.c. = 5 M.L.D.	·6	·33	·25	·2	·18
	When original toxicity per c.c. = 2 M.L.D.	·5	·25	·18	·15	·12

It will be noted that immediately after the injection the rate of disappearance is very rapid, but that later it becomes more gradual. De Croly (1897) in similar experiments found that the toxicity was unaltered five minutes after the injection, but that in two hours, a half had disappeared. Dönitz (1899), by employing another method, referred to elsewhere, also demonstrated the rapidity with which the toxin disappeared from the blood and became fixed, at first loosely, and ultimately firmly, onto the cells and tissues of the body. He found that, when seven lethal doses of diphtheria toxin were injected intravenously into a rabbit, the quantity of antitoxin which had been sufficient to neutralise the toxin when the two were injected simultaneously, was no longer able to save the life of the animal fifteen minutes after the injection of the toxin. A larger dose of antitoxin could at that period still save the animal, but after an interval of 1½ to 2 hours, no matter how much antitoxin was injected, the animal died. Heymans (1898) injected a minimal lethal dose of tetanus toxin into an animal, and immediately removed the blood which was replaced by transfusion with the blood of a healthy animal. In spite of the brief interval of time between the injection of the toxin and the removal of the blood, enough toxin had become fixed to cause the animal to succumb to typical tetanus.

The rapid disappearance of the toxin from the blood is not due to its excretion by the kidneys or intestines, for Bomstein failed to find it either in the urine or in the contents of the small intestine after the injection of large doses. Similar negative results were obtained by Goldberg (1899) in the case of tetanus toxin. Cobbett (1900) showed that, after large injections, diphtheria toxin can be demonstrated in the urine. This appears to occur only when the system is flooded with the toxin. Salter's (1898) observation that toxin may be excreted with the sweat "lacks confirmation" (Oppenheimer). According to Ehrlich the rapid removal of toxin from the blood is due to the avidity of the receptors of certain cells for the toxin. In the case of diphtheria toxin the receptors are probably widely distributed throughout the body, in the connective tissues and in various organs. Up to the present it has not been demonstrated that the cells of any organ have a peculiar affinity for diphtheria toxin such as the nerve cells have for tetanus toxin.

2. *In Refractory Animals.*

An animal may be refractory either because it has some protective substance to neutralise the toxin, or because it possesses no cells sensitive to the action of the toxin. As reference has already been made to the natural occurrence of antitoxin in man and animals and to the explanation of natural immunity from Ehrlich's standpoint, only a few words are necessary here in regard to these points. Susceptibility, according to Ehrlich, is dependent on the number and avidity of suitable receptors. These are absent or of defective avidity in refractory animals, hence the toxin has no point of attack on the body. From this it also follows that antitoxin should occur only in the blood of those normal animals which are susceptible to the action of toxin, since in these only can superfluous receptors be thrown off into the blood. This is in accordance with observation. So far the existence of normal antitoxin has been demonstrated in susceptible animals only (man and horse). As a general rule only susceptible animals furnish antitoxin in large amount as the result of immunisation. Wassermann and Futaki's observation on the combining power of brain substance with tetanus toxin is of interest in relation to this subject.

It has been shown by a number of observers (Wassermann, Futaki, Metchnikoff, and Asakawa), that the combining power of the emulsified brain of an animal is directly proportional to the *susceptibility* of that animal to tetanus toxin. For example, the brain of the susceptible guinea-pig has a powerful combining action, that of the more refractory domestic fowl has a weak combining action, whereas the brain of the turtle which is altogether refractory has no combining action.

As might have been anticipated from these results the toxin remains circulating unaltered in the blood of such refractory animals for a long period. Metchnikoff found that tetanus toxin injected subcutaneously or intraperitoneally into the marsh turtle (*Emys orbicularis*) passes into the blood and persists there for a long time. In one case the blood of a turtle four months after it had been injected produced tetanus in mice. Notwithstanding the long period of contact with the animal body antitoxin is never produced in these animals. Similar observations were made in certain fishes and in the scorpion. The toxin appears in such cases to be eliminated by a slow process of metabolism. The alligator (*A. mississippiensis*), which is also refractory to tetanus toxin, if kept at 37° C., develops antitoxin very rapidly even within 24 hours, whereas at lower temperatures it gets rid of the toxin but produces no antitoxin. The explanation of the condition in this case is difficult on Ehrlich's hypothesis. In certain cold-blooded animals such as the frog (Morgenroth), and in hibernating animals such as the marmot (Billinger and Meyer), the haptophorous group combines with the receptors at the low temperature, but the toxophorous group comes into action only when the temperature is raised.

3. *The Behaviour of Toxin in the Alimentary Canal.*

Most of the toxins fail to produce toxic effects when introduced into the healthy alimentary canal. In the case of diphtheria toxin this fact was first demonstrated by Roux and Yersin (1889).

The toxins of diphtheria, tetanus, cholera, etc., injected *per os* or *per rectum*, even in enormous quantities amounting to many thousand multiples of the lethal dose, produce no effect on the experimental animals. Snake venom (Weir Mitchell and Thomas Fraser), ricin (Ehrlich), and botulinus toxin (van Ermengem) may be mentioned as exceptions to the general rule, since they act when introduced into the stomach. Gibier (1896) showed that diphtheria and tetanus toxins injected *per rectum* produced no toxic action in the rabbit, dog or guinea-pig and that repeated doses did not give rise to immunity. Cassin and Charrin (1895) state that the internal coat of the intestine exercises a protective function against the action of certain toxins and that mere contact may modify the toxin. Ransom (1898) found in the case of tetanus toxin that guinea-pigs which had received 300,000 lethal doses by the mouth or about half that dose by the rectum suffered no apparent harm, and Nencki, Sieber and Schoumow-Simanowski (1898), working with diphtheria toxin, found that guinea-pigs which received by the stomach 600 lethal doses of the toxin remained quite unaffected. Rabbits which were injected in the same manner with 100 lethal doses suffered from a slight illness and wasting from which they completely recovered; it is suggested that the illness was perhaps due to other causes.

The reason of this harmlessness of the toxin when introduced into the gut has been the subject of much investigation. Ransom, working with the enormous doses of toxin mentioned, came to the conclusion that a large part of the toxin passed through the gut unaltered and was excreted with the faeces. He gave a guinea-pig *per os* a dose of tetanus toxin sufficient to cause the death of 25 million grammes weight of mice and found in the faeces, passed soon after the injection, sufficient toxin to kill 15 million grammes weight of mice. From these and similar results he concluded that about three-fifths of the toxin introduced *per os* escaped unaltered with the faeces. Nencki, Sieber and Schoumow-Simanowski, on the other hand, working with rather smaller doses of tetanus toxin, failed to confirm the observations of Ransom on tetanus, neither could they succeed in demonstrating the presence of diphtheria toxin in the faeces. Similar negative results were obtained by Répin (1895) and Carrière (1899).

Diphtheria and tetanus toxins introduced into the alimentary canal do not excite the formation of antitoxin (Ransom, Carrière). Notwithstanding Ransom's results most observers think that a considerable destruction of toxin takes place in the alimentary canal, and the factors giving rise to this destruction have been the subject of much investigation. Répin (1895) found that some of the digestive fluids possess a marked destructive action on toxins, both *in vitro* and in the intestine. He attributes the failure of toxins to produce their toxic effects partly to their non-dialysable character, for he found that when introduced into a ligatured loop of gut a large proportion was still present after 12 hours. Charrin (1898) observed that diphtheria toxin submitted to the action of pepsin underwent a destructive digestion or attenuation, and that the hydrochloric acid had only a slight influence in this action—the precipitate obtained by the action of the hydrochloric acid had only a feeble toxicity. Nencki found that the sterile filtrates from emulsions of the stomach, and small and large intestines, had a distinct, but by no means constant, destructive action on diphtheria toxin. The most active filtrate was obtained from the small intestine. Saliva, mucus, and filtered extracts of the lungs, liver and brain, and suprarenal bodies were inactive. Pepsin was active, the acid reaction playing little or no part as was shown by neutralisation. Pancreatic juice is the most important and active of the secretions in its action on diphtheria toxin. Charrin and Levaditi (1899) studied the action of the pancreas on diphtheria toxin. The same volume of toxin was injected into pieces of muscle and pancreas of equal weight, and after being kept at 37° C. for 10 hours the toxin was extracted and tested. The extract from the muscle was still toxic for guinea-pigs, whereas that from the pancreas had lost its toxicity. Carrière (1899), using the ferments, obtained results in the case of tetanus which correspond in the main with those of Nencki, the pancreatin being the most powerful agent. Ptyalin, pepsin, and especially bile had a considerable attenuating power; the intestinal epithelium and the intestinal microbes produced very slight attenuation. The leucocytic oxydases caused a notable attenuation. Cano-Brusso (1901) attributes to the mucous membrane of the alimentary canal a destructive action on toxins. He employed tetanus toxin in his experiments, and the results obtained pointed to a relation between the destructive activity of the mucous membrane and the susceptibility of the animal. For example, there was marked destruction in the case of the susceptible rabbit and guinea-pig, whereas in the refractory dog, cat and hen no action could be demonstrated. Baldwin and Levene (1901) found that pepsin, trypsin and papain do not merely neutralise the toxin but actually digest it. From this they argue that the toxin must be of proteid nature, since proteolytic ferments do not affect non-nitrogenous substances.

These results in regard to the behaviour of the toxin in the alimentary canal may be briefly summarised as follows. The absence of action of toxin when introduced in this manner may be due to a number of causes. The fact that toxin does not pass through an unbroken animal membrane has an important bearing on the subject. This alone is not a sufficient explanation, for certain toxins, though incapable of diffusing through animal membranes, are still capable of producing toxic effects from the alimentary canal. A great destruction of toxin must take place in the alimentary canal, for, in the case of moderate and even large doses, it frequently occurs that no toxin can be demonstrated in the faeces. This disappearance is due to a number of factors which are probably of varying importance according to the toxin employed and the conditions of the experiment: 1. the intestinal microbes, 2. the cells, 3. the secretions passing into different parts of the canal. These cause a great destruction of toxin, the bulk of the evidence being in favour of the pancreatic secretion constituting the most powerful destructive agent in the case of diphtheria toxin. The first two are probably the least important.

Action of Toxin on other Mucous Membranes.

To ascertain the action of diphtheria toxin on the mucous membranes of the respiratory tract, Roger and Bayeaux (1897) employed the method of intratracheal injection in rabbits. In some of the animals false membranes were produced on the glottis and trachea giving rise to dyspnoea. From the results obtained by them they concluded that diphtheria toxin is capable of producing false membranes without previous lesion of the mucosa.

Copez (1897) investigated the influence of diphtheria toxin on the conjunctiva. He employed a single instillation and, failing to obtain any pathological effect, concluded that the toxin cannot produce lesions if the corneal epithelium remains intact. Morax and Elmassian (1898) instilled dilute diphtheria toxin (1 in 5) every three minutes for eight to ten hours into the conjunctival sac of rabbits. They obtained all the pathological effects of diphtheria toxin, including the formation of false membrane. Some of the animals died of acute toxaemia, others developed cachexia accompanied by loss of weight.

CHAPTER XVI.

THE FORMATION OF ANTITOXIN IN THE BODY.

Discovery of antitoxins. Active immunisation. Phenomena which occur during immunisation. "Marche ondulatoire." The technique of immunisation in animals (1) with toxin modified by (*a*) chemical agents, (*b*) heat, (*c*) antitoxin (toxone), (2) with toxin only. Discussion of the value of subcutaneous and intramuscular, compared with intravenous, injection of toxin. The period of maximum concentration of antitoxin in the blood. The operation of venesection. Variability in the capacity of horses to produce antitoxin. Subsequent history of diphtheria-antitoxin horses. Active immunisation in man. The effects of various chemical and physical agents on diphtheria antitoxin; Heat, Low temperature, Light, Gases. Filtration. The testing of antitoxin. Ehrlich's method. The French methods. Attempts at the chemical isolation or concentration of antitoxin. The mechanism of antitoxin production. Ehrlich's theory.

The Discovery of Antitoxins.

THE treatment of every disease, to be scientific, must be preceded by a knowledge of its pathology including its causation. The discovery of antitoxin followed a series of events each of which formed a necessary link in the chain. We need only refer to Pasteur's epoch-making fundamental work on the causation of infective diseases which formed the basis of bacteriology and immunity, and Koch's extension of that work by which it became more and more an exact science. From an early period in the history of immunity there has been a tendency for investigation to be divided into two schools, the one dominated by the idea that the body fluids are chiefly concerned in the protection of the organism, the "humoralist's" view; the other, which attributes the most important action to the cells, the "cellular" view, of which the chief supporter is Metchnikoff. More recent work has shown that these views are not necessarily antagonistic as was thought at one time.

The work of Metchnikoff and his school, on which is based the theory of phagocytosis, has done much to make clear the phenomena which take place in the struggle between the invading microbes and the body of the host. According to this view, immunity depends on the activity of the phagocytes, which have the power of moving towards and englobing microorganisms, which they afterwards kill and digest. The phagocytes have a positive chemiotaxis to the substances secreted by the microbes, and can themselves produce substances which are capable of preparing the bacilli for phagocytosis. The cells which have these functions are mesoblastic in origin and include most of the leucocytes (with the exception of the mast cells and lymphocytes), the endothelial cells, connective tissue cells, certain cells in the liver, and so on. The wonderful manner in which the evolution of the process is traced from a simple act of nutrition in the lower forms of animal life to a complex protective mechanism in the higher vertebrates, the ingenuity and wide range of the experiments, and the beautiful simplicity of the conception make Metchnikoff's work one of the most remarkable achievements of biological science. The main theses of the hypothesis are now almost universally accepted.

Another group of workers, devoting their attention mainly to the body fluids, including the blood serum, obtained results which in the course of their development led to a series of discoveries of the highest importance in the field of immunity. Fodor (1887), Nuttall (1888), Nissen (1889), Behring (1888–89), and Buchner (1889, 1890–3), discovered that the blood serum had certain bactericidal properties of a specific character. The bactericidal substance in normal serum was named by Buchner "alexin." It was shown by Nuttall to be thermolabile, being destroyed by an exposure of half an hour to a temperature of 55—60° C. It was soon observed that the serum of naturally immune animals had in some cases a more powerful bactericidal action than the serum of susceptible animals.

The next great step was the observation made by Nissen and Behring that the serum of artificially immunised animals possessed this property in a very marked degree as compared with the serum of normal animals. They immunised guinea-pigs with the *Vibrio Metchnikovi*, and found that the serum of the guinea-pigs so immunised was strongly bactericidal in contrast to the serum of normal guinea-pigs. Bouchard (1890) made the same observation in the case of the *Bacillus pyocyaneus*.

In certain cases of acquired immunity no bactericidal substance could be demonstrated in the blood, and thus attention was directed to a condition the study of which ultimately resulted in Behring's discovery of antitoxic immunity. The discovery and isolation of the *Bacillus diphtheriae*, the specific cause of the disease, by Loeffler (1884, see Chapter I, p. 28) prepared the way for a minute study of the disease, which resulted in a thorough knowledge of the pathogenic processes involved. Roux and Yersin (1888) first succeeded in obtaining toxin from the diphtheria bacillus in broth culture, and Brieger and Fränkel (1890) published confirmatory results.

The demonstration of the possibility of obtaining these toxins in culture media formed a link in the chain of events, the importance of which in relation to immunity was widely recognised, and resulted in a number of workers in Koch's laboratory attacking the problem of immunity to diphtheria. Fränkel (1890) succeeded in obtaining immunity by means of toxins which had been heated to 60° C. and Behring (1890) by means of toxin to which iodine trichloride had been added.

From this brief outline of the history, we can see how the psychological moment had arrived for the discovery of antitoxic immunity. Behring and Kitasato in 1890 published the results of their experiments on diphtheria and tetanus, and in this publication announced the important discovery that an animal, immunised against tetanus or diphtheria, produces in its blood substances which are capable of neutralising the toxic action of the specific poison; that the blood serum, when injected into other animals before the toxin, can prevent the toxic action, and when injected even after the onset of symptoms can save the life of the animal. This substance, "antitoxin," was shown to possess neutralising, preventive and curative properties not only against the toxin but also against the infecting bacillus. The sera of the normal animals which they tested did not possess these properties. Though the results published by Behring and Kitasato concerned both tetanus and diphtheria, Behring (1893) in his history of diphtheria makes it clear that the discovery was first made by himself in the case of diphtheria, and that only after the diphtheria experiments were completed was the experience obtained from them applied by Behring and Kitasato conjointly to the subject of tetanus.

This work of Behring's must be regarded as the foundation of serum therapeutics, though the idea of using the serum of immune animals as a curative agent in other animals had occurred to a number of workers. Among those for whom a claim of priority has been made may be mentioned Héricourt and Richet (1888) who believed that the blood serum of a dog, which had received injections of *Staphylococcus pyosepticus*, protected rabbits against that organism, and Babes and Lapp (1889), who showed that the serum of dogs immunised against rabies contained substances which, when injected into other dogs, were capable of protecting them against rabies. These investigations are chiefly interesting as containing the germ of a great idea which came to fruition in Behring's work.

Behring (1893) gives a list of the methods of immunisation which were employed during this experimental stage of the investigation:

1. The treatment of guinea-pigs with bouillon cultures of the diphtheria bacillus sterilised by heat (C. Fränkel).
2. The treatment of guinea-pigs with broth cultures of the diphtheria bacillus treated with iodine trichloride (Behring).
3. The treatment of guinea-pigs with the body fluids of animals (living or dead) infected with diphtheria (Behring).
4. The cure of guinea-pigs infected with diphtheria by local treatment with different chemical agents (Behring).
5. The preparation of guinea-pigs and rabbits with hydrogen peroxide.
6. The preparation of guinea-pigs by means of a combined method with the object of reaching a high degree of immunity. In this case the animals were first treated with weakened cultures and afterwards with gradually increasing virulent cultures, or with unmodified toxin (Behring and Wernicke).
7. The treatment of rabbits by the subcutaneous injection of a heated precipitate of calcium containing diphtheria toxin (Behring and Wernicke).
8. The treatment of dogs with increasing doses of an unmodified diphtheria toxin and with unmodified broth cultures of *B. diphtheriae* (Wernicke).
9. The feeding of guinea-pigs, rabbits and dogs with diphtheria toxin (Behring and Wernicke).

For the immunisation of larger animals, such as sheep, Behring (1895) stated that he preferred the combined method.

These fundamental results obtained by Behring, Kitasato, C. Fränkel, and Wernicke soon led to the subject being carried beyond the experimental stage. Behring and Boer, Schubert and Canon, and Kossel and Korte tested the serum on children with favourable results. Aronson (1893–4) prepared a serum which gave excellent results when used on patients by Katz and Baginsky. It was, however, only after Roux's remarkable work first published in September 1894 at the International Medical Congress at Budapesth, that the antitoxin treatment of diphtheria became established on a firm practical basis. Roux in his address, and in his work published in collaboration with Martin and Chaillou (1894), gave such a clear exposition of the methods and the results that a very wide interest and enthusiasm were aroused. In this work Roux gave exact details as to the methods of growing the bacillus and obtaining the toxic filtrates. The processes involved in immunising the horses, in obtaining the serum, the result of testing the serum experimentally on small animals, and the influence of the treatment on patients were all stated in the most lucid and precise terms. Indeed, the work of Roux and his colleagues was so complete that it left room for improvement and investigation in little else than detail.

ACTIVE IMMUNISATION.

The active immunisation of animals and man is carried out by the repeated injection into the body of toxin, or of toxin modified by the action of physical or chemical agents or by antitoxin. A preliminary passive immunity may be conferred by the use of antitoxin. In rare cases the unfiltered cultures are employed. A discussion as to the relative value of the different procedures will follow under the heading "Technique of Immunisation" (p. 506).

The Phenomena which occur during Immunisation.

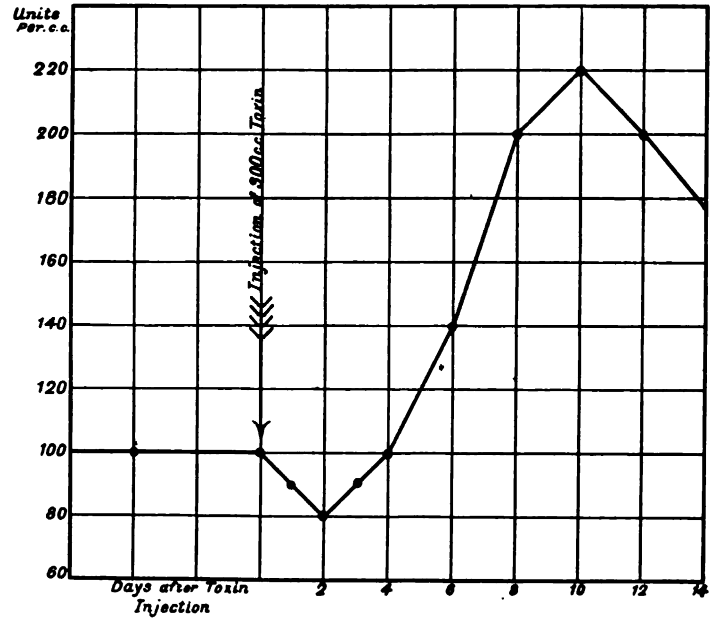
At the beginning of the immunisation the injection of toxin into the horse, goat, etc., is in many cases followed by swelling, oedema, and tenderness at the site of inoculation, with rise of temperature, loss of appetite, and the appearance of general malaise. These phenomena last for a few hours or days, according to the amount of toxin injected and the sensibility of the particular animal to the toxin. Sometimes in the horse, throughout the whole process of immunisation, the effects produced are of the most trivial character, the animal remaining apparently unaffected and gaining weight whilst under treatment.

The study of the rise and fall in the amount of antitoxin in the animal body during immunisation was first undertaken by Brieger and Ehrlich. From the investigation of the milk of a goat immunised against tetanus, they demonstrated how the antitoxin production was influenced by toxin injections in an actively immunised animal. When the antitoxic value was plotted out as a curve its undulations were found to assume a definite form. The relation of the fluctuations in the amount of antitoxin in the blood to the injection of toxin was further studied in other immunisations, for example, by Salomonsen and Madsen in diphtheria, by Morgenroth in rennin, and by Forssman and Lundstrom in botulism.

In all the cases of immunity against toxins, which have hitherto been investigated, these fluctuations have borne a general likeness to one another, the resulting curves being of similar configuration, so that they appear to obey a general law. Salomonsen and Madsen were able to study the "marche ondulatoire" of the immunisation in the case of a milch mare, which had been immunised against diphtheria toxin. They ascertained the antitoxic value of the blood serum and of the milk, and were able to confirm fully the results previously obtained by Ehrlich and Brieger.

Four phases can be distinguished in the fluctuations following a toxin injection (see Chart 1):

Chart 1, *showing the effect of a Single Toxin Injection on the Antitoxin Curve. From the* 10*th day there is a fall at first rapid, later more gradual, the curve ultimately running almost parallel to the base line, as in the position seen to the left before injection.*



First Phase. Immediately after the injection of the toxin there is a fall (or negative phase) in the antitoxic value of both the blood and the milk. This lasts for two or three days and is followed by the second phase.

Second Phase. During the second phase the antitoxic value rises till it attains a higher level than it possessed before the injection of toxin. The maximum of the rise is reached about the tenth day after the injection and is probably of very brief duration.

Third Phase. During the third phase the antitoxic value begins to fall, at first rapidly, later more slowly.

Fourth Phase. During the fourth phase the antitoxic value reaches a fairly constant level or position of equilibrium, during which the curve runs almost parallel to the base line.

Salomonsen and Madsen drew attention to the interesting point, that the fall in the antitoxic value occurring immediately after the injection of the toxin could not be accounted for by the neutralisation of antitoxin by the toxin injected. In one case, for example, the injection of 1 litre of a particular toxin caused a fall in the antitoxic value from 100 to 65 units per c.c. Now 3·8 c.c. of the blood of this horse if mixed *in vitro* with a litre of the toxin would have completely neutralised it. In a later communication Salomonsen and Madsen believed they could demonstrate a similar fall in the antitoxin of the blood by the administration of morphine, and an increase by the administration of pilocarpin; results which supported their views in regard to a negative phase and to the secretion of antitoxin by the cells.

THE TECHNIQUE OF IMMUNISING OF ANIMALS.

1. With toxin modified by (*a*) chemical agents, (*b*) heat, (*c*) antitoxin (toxone).
2. With toxin only.

1. *Immunisation with modified Toxin.*

(*a*) *Chemical agents.* In some laboratories the method of modifying the toxin by iodine trichloride is still used during the early stages of the immunisation.

(*b*) *Heat.* A modification of the method first successfully employed by C. Fränkel was suggested by Cartwright Wood (1896), who grew the bacillus on a mixture of serum and broth, filtered the culture through a Pasteur-Chamberland candle, and heated to 65° C. for one hour. The horses received large doses of this fluid, 380 c.c. in three doses in the first twelve days. It was claimed for this method that it was safe and rapid and that the toxin was essentially different from ordinary toxin. It is probable, however, that we are dealing either with toxoids or with a toxin which has undergone a fractional destruction. After a considerable experience of this method the writer abandoned it for the method described later (p. 509).

(c) *Toxin modified by, or employed together with, antitoxin.* After the demonstration of the wonderful preventive power of antitoxin, it naturally occurred to a number of workers that the dangers attending the early stages of immunisation might be averted by the use of antitoxin given before or at the same time as the toxin, or as a toxin-antitoxin mixture.

Nikanorow (1897) found in the case of two goats that, under the same conditions, the one which received toxin on one side of the body and antitoxin on the other side gave an antitoxin value of 45 antitoxin units, whereas the other, which received only toxin, gave 15 antitoxin units. He employed also, in the case of the horse, a number of injections of antitoxin before beginning the treatment with toxin, and claimed that one could thus obtain rapidly and safely a very high antitoxic value.

Kretz (1901) in the course of a study of the paradoxical phenomenon, that an animal may die of toxaemia and yet have a large quantity of antitoxin in its blood, performed several immunisations, which are of interest in relation to the influence which antitoxin injections may exert on the production of antitoxin by the animal body. The results obtained in the case of four horses were as follows:—

Horse	Method of administering toxin and antitoxin	Method of injection	Resulting value of antitoxin
1.	Toxin and antitoxin mixed at room temperature for 18 hours	Intravenous	>2 A.U.[1]
2.	Do. do. do.	Subcutaneous	=8 A.U.
3.	Same quantity of serum. Toxin 12—15 hours later	Intravenous	=c. 20 A.U.
4.	Do. do. do.	Subcutaneous	+100 c. 500 A.U.

It would thus appear that hyper-neutralisation of the toxin is unfavourable to antitoxin production, whereas preventive injection of antitoxin at least allows antitoxin production to proceed. Whether it hinders or aids cannot be ascertained from these experiments. Dzierzgowski (quoted by Eisenberg, 1903) failed to obtain any antitoxin by injecting neutral mixtures of toxin and antitoxin into four goats, two dogs and two horses.

Arloing, Nicolas and Antoine (1901) investigated the antitoxin production in dogs injected with mixtures of antitoxin and toxin, or antitoxin and bacilli. The antitoxic value of the blood serum was so low that it could be altogether accounted for by the antitoxin injected.

---

[1] A.U. = antitoxin units (see p. 521).

Arloing and Nicolas (1901) carried these experiments further, using the ass as the experimental animal. In one case, after a long treatment, a very weak antitoxin was obtained by the simultaneous injection of antitoxin and toxin in different parts of the body. In a second experiment they used three animals. The results of these experiments are shown in tabular form below.

Animal	Immunised with	Antitoxic value of the serum per c.c.	Preventive strength
Ass 1	Toxin alone: 131 c.c.	80 A.U.	1 : 50,000
Ass 2	Toxin and antitoxin at different places: 446 c.c. toxin 149 c.c. serum	60 A.U.	1 : 5,000
Ass 3	Mixture of toxin and antitoxin as above	10 A.U.	1 : 500

N.B. The antitoxic value of the serum was ascertained 20 days after the last injection.

It is clear from this table that the best results are gained by the use of toxin alone; where the toxin and antitoxin are injected at separate parts of the body a fairly high antitoxic value is reached; the poorest result is that obtained by the use of a toxin-antitoxin mixture.

Atkinson (1903) gives the results obtained in a series of 100 horses. Over-neutralised toxin-antitoxin mixtures were employed in the first stages, and four months were required to reach a maximum antitoxic value. The doses were repeated every five to seven days, and were increased each time by a quarter to a half. By this method one half of the horses develop sera of 300 units per c.c., and one-fourth develop sera of 500 units per c.c. or more.

Madsen and Dreyer (1901) by immunising with mixtures of toxin and antitoxin in which only the toxone was free succeeded in obtaining antitoxin; an observation of great importance from the theoretical standpoint.

Since the publication of the work of Dzierzgowski and Kretz, Morgenroth has shown that the union between diphtheria toxin and antitoxin is not so rapid as had been imagined, consequently it seems probable to the writer that the slight antitoxin production is due to a small amount of free toxin reaching the necessary cells, before combination has had time to occur. The same argument can be applied to the case where antitoxin is given as a preventive inoculation at the beginning of immunisation as in the work of Kretz and others. If this view be correct it has yet to be proved that the previous injection of antitoxin is of any advantage. The writer, from personal observation, has a strong impression that the injection of antitoxin in the early stages of immunisation is unfavourable to the attainment of a very high antitoxin production. These considerations, and those dealt with in the following section, led the writer to adopt a method of immunising with unmodified toxin alone.

2. *Immunisation by Toxin alone.*

The employment of toxin alone as the immunising agent in the preparation of diphtheria antitoxin has been in wide use since the publication of Behring's and Roux's early results.

The methods just described have been introduced with the view of avoiding the risks attendant on the process of immunisation, especially during the first stages, or in the hope of shortening the period necessary to obtain antitoxins of sufficient strength to be of practical value. So far as the writer's experience goes an immunisation which is both comparatively safe and rapid may be attained by the use of toxin only. Safety may be secured by beginning with very small doses of toxin. The chief difficulty in this respect is the varying susceptibility of individual horses. An interesting example of this occurred in his experience. Three horses each received 1 c.c. of a particular toxin as a first injection. One of the three died within five days with all the signs and symptoms of an acute toxaemia, whereas the other two were so little affected by the first injection that they had already received their second injection before the death of the first horse. After this experience the first dose was reduced to 0·1 c.c. but even this was found to be too high for certain animals, so that now an initial dose of about 0·01 c.c. is employed.

In regard to the *frequency* with which the injections ought to be repeated, reference must be made to the antitoxin curves of Ehrlich and Brieger, and Salomonsen and Madsen (p. 505). From their curves the authors naturally deduced that the most favourable time for repeating the injection of toxin occurs when the curve has reached its maximum point. It would seem at first sight that fresh injections at these times would lead to an almost indefinite rise in the antitoxic strength of the blood. As first pointed out by the writer (Dean, 1899) however, the highest antitoxic values are not reached by the procedure based on this theory. Higher antitoxic values are attained by injecting the animals within the first three days after the previous injection, that is, during the period of fall of the curve or negative phase. It would seem as if this must result in a summation of negative phases ultimately causing toxaemia and death. This does not occur, however, and the opinion expressed in the communication, to which reference has been made, has been confirmed and strengthened by the results obtained with more than fifty horses.

To get a correct idea of the events which probably occur during this method of immunisation it is necessary to form a mental picture of a summation, or super-imposition, of curves. Madsen explains the better results which are obtained by injecting every third day instead of on the day when the antitoxin curve is supposed to reach its greatest height, in the following words:

> "The period of production, on an average nine days, can approximately be divided into three periods: three days of incubation, thereafter three to four days to the maximum of production, and lastly two to three days to the end. Thus, an injection given three days after another will have its maximal production at the time when the effect of the first has ceased. In this way no interruption in the increase of the antibody will take place. If the injection is given on the day of the acme, six days or so will pass before the maximum of production is reached, and during those six days a considerable loss of antibody occurs."

The form of curve obtained during such an immunisation was very thoroughly studied in the case of one horse "B," complete charts of the early stages of the immunisation of which are given (Charts 2 and 3). The antitoxic value of the serum of the horse was tested before immunisation, and immediately before every injection a small sample of blood was taken from the jugular vein and its antitoxic value tested. It would be impossible to study the curves of many animals so minutely as was done in this case, seeing that each point in the curve requires for its determination from four to 10 guinea-pigs. The result is not exceptional as the antitoxin in many of the horses reached a higher value in the same time (see Table, p. 514) than is recorded in the case of this animal[1].

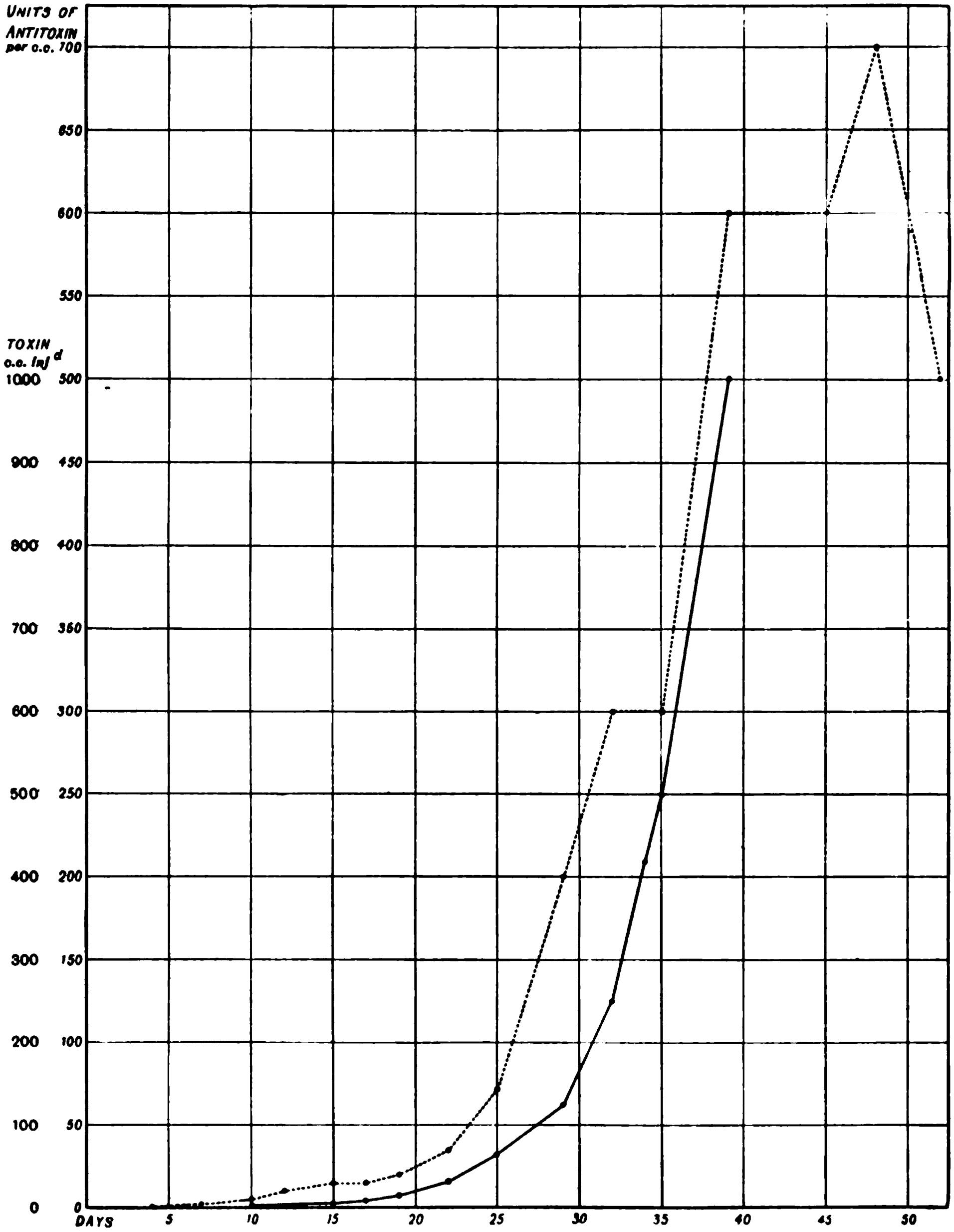
Before a fresh injection the swelling, stiffness, slight loss of appetite and rise of temperature resulting from the previous injection must all have disappeared. In the later stages of immunisation slight swelling at the site of injection may be disregarded.

This method of rapid immunisation does not give a larger number of accidents than any other, and has the advantage of giving a higher

---

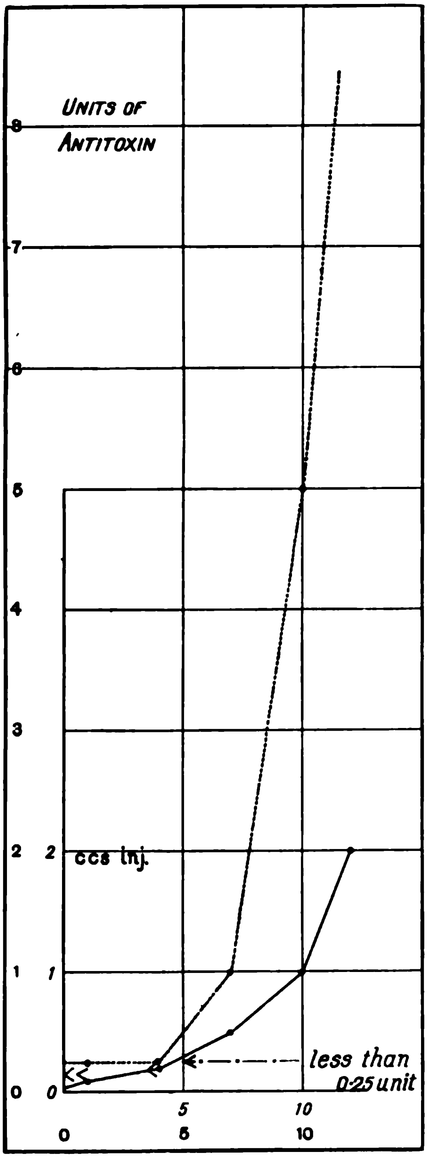
[1] Since this was written Dr T. C. G. Ledingham (1907) has worked out in the Lister Institute and published a similar complete antitoxic curve obtained by the same process of immunisation.

Chart 2. A typical Antitoxic Curve obtained by the rapid method of Immunisation.



The dotted line indicates the number of antitoxic units per c.c., and the uniform line the quantity of toxin injected.

Chart 3, showing the first stages of Chart 2 on a larger scale.



Dotted line indicates antitoxic units per c.c., uniform line volume of toxin in c.c.

antitoxic value in a very short time; both matters of great practical importance.

The essential points of the method, which the writer has found most useful in immunisation, are:

1. The employment of pure toxin only.
2. The injection during the initial stages of only small doses beginning with say 1/100 c.c., very frequently repeated.
3. The choice of the period of negative phase for the injection, if possible on or before the third day after the previous injection.
4. The increase in the dose over the previous dose must be regulated but is usually 50 to 100 %.
5. By this method when a final injection of say 1,000 c.c. has been reached, the antitoxic value is found in many cases to have attained its maximum. This occurs within a period of seven to eight weeks.

The following Table gives the dates and doses of a typical immunisation.

Type of Immunisation

May 31st	0·01 c.c. Toxin[1]	June 17th	15 c.c. Toxin
June 1st	0·02 ,,	,, 20th	30 ,,
,, 3rd	0·1 ,,	,, 22nd	50 ,,
,, 4th	0·25 ,,	,, 24th	100 ,,
,, 6th	0·5 ,,	,, 27th	200 ,,
,, 8th	1·0 ,,	,, 30th	400 ,,
,, 10th	2 ,,	July 4th	500 ,,
,, 13th	4 ,,	,, 7th	1000 ,,
,, 15th	8 ,,		

---

[1] This toxin kills guinea-pigs of 250 grammes weight in a dose of 1/400 c.c. The early injections were pushed on rapidly in this case as they gave rise to no local or general reaction.

The results obtained in a continuous series of 57 horses are shown in the following Table. In comparison with other methods the mortality is not excessively high; three horses out of the 57 having died of acute diphtheria toxaemia, an acute toxaemia mortality of 5·3 %.

Thirty-eight out of 54 survivors (70·4 % or 66·7 % of total number) produced antitoxin having an antitoxic value above 500 antitoxic units at some period of the immunisation.

Table of results obtained by the writer using the rapid method of immunisation in a continuous series of 57 horses.

Units of antitoxin	No. of horses	Percentage of horses with units indicated	
		On surviving horses	On total horses
1000	7	13	12·3
900	7	13	12·3
800	2	3·7	3·5
700	1	1·9	1·8
600	10	18·5	17·5
500	11	20·4	19·3
400	9	16·6	15·8
300	5	9·2	8·8
200	1	1·9	1·8
less than 20	1	1·9	1·8

Three animals died of acute diphtheria toxaemia.

DISCUSSION ON THE VALUE OF SUBCUTANEOUS OR INTRAMUSCULAR COMPARED WITH INTRAVENOUS INJECTION OF TOXIN.

Cobbett (1896) from his experience suggested that good results might be obtained by a combined method of subcutaneous and intravenous injection. It seems certain, however, that the method of subcutaneous injection is superior to the intravenous. Dzierzgowski (1902) indeed, from a very wide experience, holds that the injection of toxin into the blood stream does not contribute to antitoxin production. He believes that the toxin injected intravenously is at once neutralised as it would be *in vitro*. In the case of subcutaneous injection only a small part diffuses into the blood, the bulk becomes attached to the cells of the subcutaneous tissue which are then stimulated to antitoxin production.

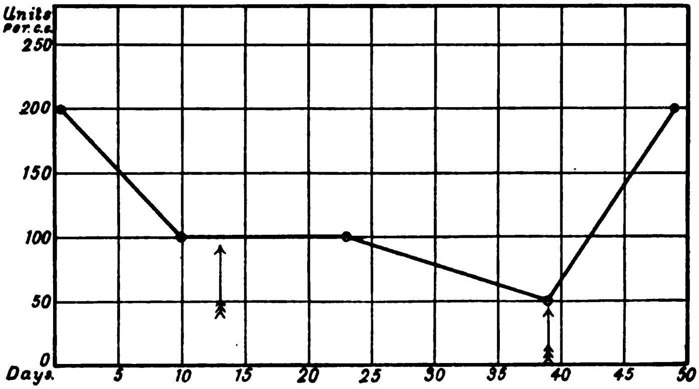
According to this view the production of antitoxin is a process which occurs locally at the site of injection. There is much to be said in favour of this local production of antitoxin. One of the strongest arguments is an experiment by Römer (1901) who showed that if a toxin was repeatedly injected into the conjunctiva of one eye of an animal, that conjunctiva, at a certain stage, could neutralise a given dose of toxin, whereas the other unimmunised conjunctiva possessed no such neutralising properties.

Forssman (1905) investigated the question as to whether antitoxin production resulted from the intravenous injection of botulinus toxin.

He found that intravenous injection did give rise to antitoxin formation but that, whereas the antitoxin curve resulting from subcutaneous injection reached its maximum on the fifteenth day, the curve resulting from intravenous injection was highest on the tenth day. Forssman does not believe that toxin and antitoxin interact in the same way in the animal body as *in vitro*. He shows from Dzierzgowski's own figures that there is evidence of antitoxin production though to a very slight extent.

The writer's experience is limited as to the value of intravenous injection in diphtheria immunisation. In the case of three horses, where a certain rise in the antitoxin curve might have been anticipated as the result of a subcutaneous injection, an intravenous injection of toxin was given. This was followed by little or no rise in the antitoxin curve. The subsequent subcutaneous injection of the same dose of toxin was followed by the expected normal rise. (See Chart 4.)

Chart 4 of Antitoxin Curve before and after Intravenous and Subcutaneous Injections.



At the time indicated by the arrow on the left 1000 c.c. of toxin were injected intravenously, and at the time indicated by the arrow on the right 1000 c.c. of the same toxin subcutaneously.

THE PERIOD OF MAXIMUM CONCENTRATION OF ANTITOXIN IN THE BLOOD.

The study of the antitoxin curves (p. 505) has shown that the antitoxin reaches its maximum concentration in the blood about the tenth day after an injection of toxin. In the preparation of antitoxin, therefore, a period of from eight to twelve days is allowed to elapse after the final injection of the series before the removal of the blood.

The Operation of Venesection.

This is carried out under strict asepsis. After sterilisation of the field of operation a small incision is made through the skin over the jugular vein in its long axis, and through this a quill-cut cannula is introduced into the vein which is made to bulge by pressure. To the cannula is attached a rubber tube, by means of which the blood is conducted into sterilised glass vessels. The amount of blood withdrawn varies from 6 to 10 litres according to the size of the animal. The blood may be allowed to clot or may be defibrinated. The serum after being decanted into sterile vessels is the fluid generally known as antitoxin though the actual antitoxin present must constitute only a small fraction of the total serum.

Interval between Venesections.

At least a month is allowed to elapse between the venesections. After a short period of complete rest a few injections of toxin are given to excite the antitoxin production before each withdrawal of blood.

VARIABILITY IN THE CAPACITY OF HORSES TO PRODUCE ANTITOXIN.

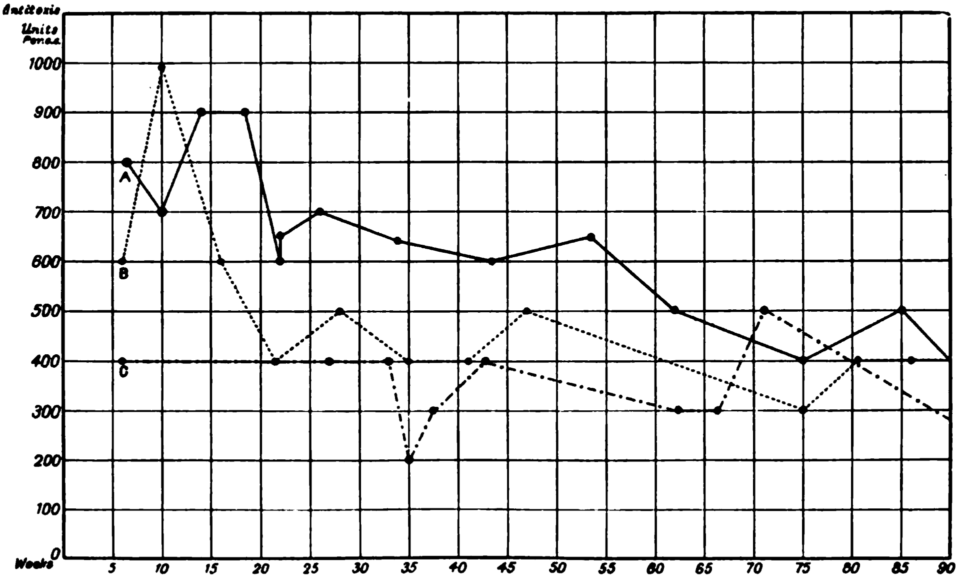
Apart from other circumstances there is no doubt that considerable individual variations occur among horses in regard to their capacity for antitoxin production.

The most striking example in the writer's experience occurred in the case of the last horse in the table on p. 514. This animal was immunised at the same time as another, the same toxin was employed, and the course of immunisation throughout was practically identical in both cases. The serum of the one animal contained 700 A.U. per c.c., whereas that of the other animal had a value of under 20 A.U. per c.c. According to Ehrlich's view this animal must have had some peculiarity or defect in his side-chains. Such a marked absence of the power of antitoxin production appears to be comparatively rare.

SUBSEQUENT HISTORY OF DIPHTHERIA-ANTITOXIN HORSES.

The period during which animals produce a high-grade antitoxin may vary from six months to several years. In the history of most, if not all, antitoxin horses, there comes a time when the injection of toxin is not followed by the usual response of antitoxin production. No matter how severe the stimulation the antitoxin curve goes steadily down. This is shown in Chart 5. If, when signs of this fall begin to appear, a rest of several months is given, the animal may at the end of that time be found to have recovered the property to a large extent.

Chart 5, showing typical Antitoxin Curves of Horses during Diphtheria Immunisation.



A and *B* are common types in the case of the higher grade horses. All the curves, especially *C*, show the tendency to fall after a time in spite of the stimulation of the animals by large injections of toxin.

Active Immunisation in Man.

The active immunisation of man in the case of diphtheria has been little practised. So far as the writer has been able to ascertain only one case has been recorded.

This was carried out in the case of a female patient, one of a group of cases of chronic disease of the upper air passages (Kahnert (1900)). In these cases virulent diphtheria bacilli were found, and the serum of the patient contained antitoxin. As usual in such chronic infections of the nose the injection of antitoxin had no effect on the pathological process. With the view of ascertaining whether an increase of the antitoxin content of the blood would effect an improvement, Kahnert, using a toxin standardised by Ehrlich, proceeded to try active immunisation. The patient received the following injections :—

On 28th March	0·005	c.c.	diphtheria	toxin
5th April	0·01	c.c.	„	„
11th April	0·015	c.c.	„	„
18th April	0·02	c.c.	„	„
30th April	0·02	c.c.	„	„
7th May	0·03	c.c.	„	„
15th May	0·04	c.c.	„	„
25th May	0·06	c.c.	„	„
12th June	0·08	c.c.	„	„

The injections caused at first painful swellings at the site of inoculation, and had to be discontinued for reasons not connected with the treatment. Whether the great improvement in the condition which occurred was attributable to the treatment could not be decided, as the better seasonal climatic conditions may have had an important influence. The state of the mucous membrane and bacteriological findings were not altered.

Such a heroic measure as active immunisation would be only justifiable in troublesome chronic cases. It is possible that the bactericidal serum suggested by L. Martin may prove useful in cases of this type.

Effect of Various Physical and Chemical Agents on Diphtheria Antitoxin.

Effect of Heat on Antitoxin.

Van de Velde (1897) found that temperatures of 60°, 65°, and 70° C. had an injurious effect on both antitoxic and antimicrobial sera. Marenghi (1897) found that serum could be heated at 55° C. in open or closed tubes for five hours and still retain its antitoxic value unaltered.

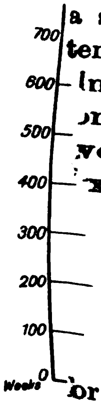
This is in agreement with Spronck's (1898) view who found that on exposure to 59° C. for twenty minutes only a very slight loss in antitoxic power occurred, whereas the substances which give rise to the unpleasant after-effects, such as skin eruptions and joint-pains, were to a large extent eliminated. Even comparatively low temperatures acting for a long period have a deleterious action. Thus Müller (1898) found that a great loss occurred when the serum was kept at 37° C. for two months.

Dry antitoxin is very resistant to external influences. Camus (1898) found that dry antitoxin resisted a temperature of 140° C. for a quarter of an hour.

Effect of Low Temperatures.

Low temperatures have no bad effects on antitoxin. Bujwid (1897) found that freezing did not lower the antitoxic value even when repeated several times. The temperature of liquid air does not diminish the antitoxic value (Macfadyen).

Bujwid (1897) believed that he could concentrate antitoxin by means of a process of alternate freezing and thawing. He states that during freezing colourless water crystals at first appear, and extend in various directions till most of the serum is frozen, with the exception of a small volume of brownish fluid. The serum, on thawing at room temperature, forms two layers; an upper clearer layer which contains almost no antitoxin, and a lower layer of an intense yellow colour, which contains almost all the antitoxin. The freezing and thawing is repeated two or three times, and in this way a strong concentration of the antitoxin results[1].



Effect of Light on Antitoxin.

Antitoxin is much less sensitive to the action of light than toxin.

Marenghi (1897) found that the Roman July sun acting on antitoxin for several days produced a bleaching of the serum but no effect, or only a very slight one, on the antitoxic value.

Müller (1897) on the other hand found that daylight was injurious. As might have been anticipated red or yellow light was much less deleterious than green or blue light.

Effect of Gases on Antitoxin.

Air, oxygen, nitrogen, hydrogen, and especially carbonic acid were all found by Müller to have an injurious action.

Reference will be made to the action of various ferments in the section on the behaviour of the antitoxin in the alimentary canal.

---

[1] The writer failed to find Bujwid's method useful in practice.

Effect of Filtration on Antitoxin.

Martini (1896) first showed that a certain loss in antitoxic value resulted from the filtration of a serum. This was denied by Dzierzgowski (1897) who found that no diminution took place. With the object of solving the question, Cobbett (1898) carried out a number of tests from which he concluded that there is an undoubted loss of antitoxin both with the Berkefeld and Pasteur-Chamberland filters. The loss depends on the size of the pores of the filter, but even in the case of the Berkefeld filter it may reach to 30% of the antitoxin present, and when Martin's gelatine filter is used all the antitoxin may be kept back (Brodie, 1897). The loss of antitoxin is progressive owing to the pores of the filter becoming more and more blocked. The writer has found that when the ordinary "V" Berkefeld filter is employed with a low pressure, the loss is so slight that from the practical point of view it is negligible. In any case the antitoxic value should be determined after and not before filtration.

When kept under moderately favourable conditions, in a dry cool place, and in the dark, diphtheria antitoxin keeps for years, as has been shown by Miller (1905) among others. The higher potency sera deteriorate rather more rapidly than the lower. (For preservation in the dried state *in vacuo* see p. 521.)

THE TESTING OF ANTITOXINS.

Ehrlich's method.

Although it is unnecessary here to give a minute account of all the phases in the evolution of Ehrlich's method of testing antitoxin which led to the method at present practised, a brief description of some of the more important stages is desirable in order to obtain a clear understanding of the basis of the method.

The method first employed by Behring of injecting antitoxin into one part of the animal and living culture into another part was soon abandoned owing to the irregularity of the results. Ehrlich, Kossel and Wassermann (1894) found that greater accuracy was attained by mixing the toxin and the antitoxin *in vitro*, and subsequently injecting the mixture into a guinea-pig, which was early selected as the most suitable animal for test purposes. The standard at this period was an old test toxin of Behring's and the test dose employed was ten times the minimal lethal dose for a guinea-pig of 200 to 300 grammes weight. The unit at that time was the quantity of serum which completely neutralised the action of ten lethal doses of the toxin, *i.e.*, prevented the occurrence of all local or constitutional effects. Later, as the antitoxins became stronger, Behring and Ehrlich, while retaining ten minimal lethal doses as the test dose of toxin, changed the *unit* to ten times the quantity of serum which would completely neutralise the ten lethal doses, that is to say, the antitoxic unit was now fixed as the quantity of antitoxin which would neutralise 100 M. L. D., for a guinea-pig weighing 250 grammes. For example, if 0·1 c.c. of a serum mixed with ten M. L. D. of the test toxin and injected into a guinea-pig behaved towards the animal body as an indifferent substance, then 1 c.c of this serum contained one antitoxic unit. If 0·001 c.c. of a serum produced the same result then the antitoxin contained 100 units per c.c.

Ehrlich at this stage undertook his epoch-making study of the diphtheria toxin, a brief outline of which has already been given (p. 473). He became convinced that both diphtheria toxins and antitoxins in the liquid form were unstable bodies, and found that attempts to use either as standards led to unsatisfactory results. For example, it was found that the neutralisation point of a particular toxin and antitoxin remained constant for more than one year, and yet on closer investigation both toxin and antitoxin were shown to have become weakened. It was observed that in certain cases the loss in antitoxin ran parallel to the loss in toxin, and this fact was especially liable to lead to error. It was therefore clear that constancy of the neutralisation point was no sure guarantee of constancy in the test solutions.

Ehrlich argued that from the chemical side the agents most likely to cause these changes were (1) water, through hydration, (2) oxygen, by causing oxidation, (3) light, and (4) heat. The last two can be easily eliminated, and he proceeds to consider how best to avoid the action of water and oxygen. Though it is possible to obtain diphtheria toxin in the dry state by precipitation with ammonium sulphate there are certain objections, as pointed out by Ehrlich, to the use of such a toxin as a standard. Owing to the presence of a certain amount of crystalline ammonium sulphate in the powder it tends to arrange itself into layers, the upper layers being more toxic than those below. Besides this, a great part of the toxin may be transformed during the process of precipitation into modified substances which are capable of interfering with the testing. As the more suitable substance to work on in the search for a standard, he therefore chose dried diphtheria antitoxin. Dried diphtheria antitoxin is put into a small glass apparatus consisting of two tubes with a connecting tube forming an "H" shaped arrangement. The antitoxin is put into one tube, and phosphoric anhydride into the other to act as a dehydrating agent. The tube containing the dried serum is sealed off and then as complete a vacuum as possible is obtained by using a mercury pump. The second tube is then sealed off and by these means the antitoxin becomes thoroughly dry in what is practically an oxygen-free atmosphere. The tubes are kept in a cool dark place. A number of tubes were prepared in this way, each tube containing two grammes of a dry serum which by the older method had been estimated to contain 1,700 immunity or antitoxic units. Every two or three months one of these tubes is carefully opened and the contents dissolved in 200 c.c. of a 10% salt solution which contains 50 to 80% of glycerine. In this way a test serum containing 17 units per c.c. is obtained. This method of preparing and conserving a standard antitoxin has given uniform and reliable results, and both for practical and theoretical purposes has proved most satisfactory. The unit derived from the older method of testing is a perfectly arbitrary one, and if serum is to be measured by this method the standard unit must first be obtained from Ehrlich. As already indicated the quantity of serum which neutralised 100 M.L.D. of a particular diphtheria toxin which was in use at that time was chosen as the antitoxic unit. Neutralisation was indicated by a complete absence of reaction, local or general, in the guinea-pig. Madsen (1902) has stated that by the physico-chemical methods employed by himself and Arrhenius it would be possible, if the standard unit were altogether lost, to recover it by calculation.

In the older methods referred to the strength of the antitoxin was determined by ascertaining the amount of antitoxin necessary to completely neutralise a certain fixed quantity of toxin. The test was a subjective one and the results were influenced largely by the personal factor. The determination of the presence or absence of slight traces of oedema was a constant difficulty. To abolish this source of error Ehrlich introduced an objective test, namely death, as the criterion in determining the strengths of antitoxins. The test dose of toxin, instead of being the L_0, was now made the L_+ dose. The latter, it will be remembered, is the amount of toxin which, mixed with one unit of antitoxin, causes the death of a guinea-pig of 250—280 grammes weight in four or five days.

The Selection of a Suitable Test Toxin.

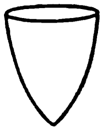
The toxin to be employed for test purposes must have reached a condition of stability. It should be stored in a cool, dark place in closely stoppered bottles of large capacity (2 litres), under a layer of toluol. In many cases a period of nine months to one year must elapse after filtration before equilibrium is established. The M.L.D., L_0 and L_+ values are then determined. It is not desirable to use for test purposes a toxin whose L_+ is over 1 c.c., nor should the difference between the L_+ and the L_0 be more than 15 M.L.D. Assuming that the toxin has been previously found suitable in regard to these points it may be used as a test toxin. Its sterility and stability must be ascertained by frequent tests. It may be desirable here to outline briefly the technique of testing.

TECHNIQUE OF TESTING.

The glass vessels, syringes, etc., employed in the process of testing should be sterilised.

The Determination of the L_+ Dose.

The determination of the L_+ dose must be done with great accuracy, and may require several series of tests. The appropriate dilution of Ehrlich's standard antitoxin solution is made containing 10 antitoxic units per c.c. One c.c. of the standard solution is pipetted from the bottle by means of a 1 c.c. glycerine pipette, and put into a flask of 50 to 100 c.c. capacity. Nine c.c. of sterilised tap or distilled water, which may be most conveniently measured by a standard burette, are introduced into the flask. The antitoxin and water are well mixed by shaking. One c.c. of this solution contains one antitoxic unit. A number of small conical test glasses, corresponding to the number of tests to be made, are arranged in a suitable stand. Into each of these test glasses 1 c.c. of the dilution of antitoxin, containing one antitoxic unit, is introduced. From a rough test previously carried out two limits, between which the L_+ dose must lie, have been ascertained. Between these two limits a series of quantities of toxin are fixed on for testing, the spacing between the doses being made as nearly uniform as possible. Each quantity of toxin, beginning with the smallest in the series, is placed in one of the test glasses containing an antitoxic unit. Each mixture of toxin and antitoxin is brought up to a volume of 4 c.c. and after standing



at room temperature for about 20 to 30 minutes is injected subcutaneously into the abdominal wall of a guinea-pig weighing 250—280 grammes. The weight and the condition of the site of inoculation are ascertained and recorded day by day, and eventually the date of the death of the guinea-pig is noted. The mixture which gives rise to the death of the guinea-pig on the fourth or fifth day after inoculation contains the correct L_+ dose of toxin. If two mixtures cause death on this day the smaller of the two doses is the L_+ dose. An example of such a test may be given:—

Test to determine the L_+ dose of Test Toxin No. 30.

Guinea-pig	Mixture injected	Result
1	1 antitoxic unit + ·34 c.c. toxin	Slight local oedema, late paralysis.
2	,, + ·35 ,,	Large local oedema; great loss in weight, death on 6th day.
3	,, + ·36 ,,	Large local oedema, death on 4th day.
4	,, + ·37 ,,	,, ,, ,, ,, 3rd day.
5	,, + ·38 ,,	,, ,, ,, ,, 3rd day.
6	,, + ·39 ,,	,, ,, ,, death in 48 hours.
7	,, + ·4 ,,	,, ,, ,, ,, 48 hours.

From this test it can be seen that the correct test dose (L_+) of this series is ·36 c.c. Previous tests gave the M.L.D. as ·005 c.c., the L_0 as ·31.

$$L_+ - L_0 = \cdot 05 \text{ c.c.} = 10 \text{ M.L.D.} < 15 \text{ M.L.D.}$$

The toxin, therefore, is suitable for test purposes.

The L_+ may now be employed for testing the antitoxic value of any serum by substituting the serum to be tested for the antitoxic unit. In this test the constant is the toxin, the variable the antitoxin. A simple example may be given of the method of ascertaining whether a serum contains one, five or ten antitoxic units per c.c.

Test of Antitoxic Serum for 1, 5 *or* 10 *antitoxic units per* c.c.

Guinea-pig	Mixture injected	Result
8	1 c.c. of the serum + ·36 c.c. toxin	Lives; no oedema.
9	1/5 ,, ,, + ·36 ,,	Large oedema; loss of weight; dies on the 4th day.
10	1/10 ,, ,, + ·36 ,,	Large oedema; dies on the 2nd day.

The serum therefore contains 5 but not 10 units per c.c.

Test of Antitoxic Serum to ascertain whether it contains 400, 450, 500, 550 *or* 600 *antitoxic units.*

Guinea-pig	Mixture injected	Result
11	1/400 c.c. serum + ·36 c.c. toxin	Slight oedema; no loss of weight, lives.
12	1/450 " " + ·36 "	" " " "
13	1/500 " " + ·36 "	Medium oedema; small loss of weight, lives.
14	1/550 " " + ·36 "	Large oedema; great loss of weight, dies on the 4th day.
15	1/600 " " + ·36 "	Large oedema; dies on the 3rd day.

This serum contains, therefore, 550 but not 600 units per c.c.

The French Methods of Standardising Sera.

The French still employ the method of testing the preventive and curative strength of a serum apart from its toxin neutralising power.

The *preventive strength* is said to be 50,000 when 0·01 c.c. of the serum protects a guinea-pig of 500 grammes weight against a dose of diphtheria toxin which kills a control animal of the same weight in 36—40 hours. The relation between the weight of the animal 500 and the quantity of serum employed is the measure of the preventive strength of the serum. The serum is injected 12 hours before the toxin. Every guinea-pig which has not lost weight after four to six days is considered protected.

The *curative strength* of a serum is estimated in a similar manner. In this case the toxin is injected six hours before the serum. The guinea-pigs surviving on the sixth day are considered cured. In place of the toxin the living bacilli may be employed for the tests.

While fully appreciating the value of Ehrlich's researches into the constitution of toxin, Roux (1900) indicates certain difficulties in accepting all his views. He points out the impracticability of determining the L_0 limit of a toxin, and shows that a mixture, which is apparently completely neutralised for guinea-pigs under ordinary conditions of temperature, is still lethal for the same animals kept in a moist, cold temperature about 2·5° C. Similarly, mixtures are still toxic for small birds though they contain more antitoxin than is necessary for complete neutralisation in guinea-pigs. It may be asked indeed if such a thing as a neutral mixture really exists. He notes also that toxic mixtures when injected before a lethal dose appear to delay the lethal period.

These observations of Roux are of great interest when regarded in relation to the recent work of Madsen and Arrhenius. A comparison of the French method and Ehrlich's method shows, according to Roux, that the latter does not give a complete, or quite accurate, index of the value of a serum.

Two sera were tested by Ehrlich's method and were found to contain, the one, "A," 700, and the other, "B," 200 antitoxin units. M. Momont found that the preventive strength of "A" was 150,000 and of "B" 200,000.

M. Danysz on testing the curative strength found that 10 units of serum "A" produced the same effect as two units of serum "B." The serum therefore, which reckoned by Ehrlich's antitoxic units was three and a half times as strong as the other, was inferior both in preventive and curative properties.

As the result of Roux's statement Marx (1901), working in Ehrlich's laboratory, instituted a series of tests to ascertain whether anti-diphtheritic sera of the most varied origin could possess antitoxic, preventive, and curative properties in varying degrees, or whether the number of antitoxic units was an index of all these properties.

He used the following antitoxins :—Ehrlich's standard dried serum, 1700 A. U., Höchst 1150, Höchst 750, Paris 175 and cow serum 25, and tested their preventive and curative action in relation to their antitoxic strength on rabbits and guinea-pigs.

He came to the conclusion that the toxin neutralising strength (the antitoxic value) of an anti-diphtheritic serum, the immunising and the curative actions are three factors which stand in the closest relation to one another, and that the immunising and curative effects of a serum are directly proportional to the number of antitoxin units contained in it. As Ehrlich's method of estimation is far the easiest and most accurate it is to be preferred to all others.

Madsen (1897), who made a comparative study of the French method and of Ehrlich's earlier method, came to the conclusion that the latter gave the more accurate results. It must be remarked, however, that in this study Madsen was not considering the possibility of sera having different properties in different relative proportions.

The author carried out a series of experiments with the view of ascertaining whether the antitoxic content of the sera of horses at different stages of their immunisation gave an accurate measure of the preventive and curative properties as tested by the French method. The sera selected for the tests were from three horses, one of which, newly immunised, was at the height of its antitoxin production, and had 1000 A.U. per c.c. in its serum; the second horse was at the stage when the antitoxin curve, in spite of large injections, was steadily falling, the serum containing 300 A.U. per c.c.; the third horse had received no toxin injections for several months; its serum contained 100 A.U. per c.c.

He concluded that the estimation of the antitoxin content by Ehrlich's method gave in the case of these three sera a correct measure of all three factors, the neutralising, the preventive, and the curative. His conclusions, therefore, agreed with those arrived at by Marx.

Cruveilhier (1905) has recently repeated the experiments on which Roux based his opinions, and has fully confirmed them. He employed for his tests living cultures of various origin, and tested sera of known antitoxic strength both for their preventive and curative properties. He concluded that these tests were of great value in estimating the therapeutic value of a serum, and that the usual test for antitoxic strength does not give a correct indication of the efficiency of a serum.

Attempts to chemically isolate or concentrate antitoxin.

Brieger and Ehrlich (1893) attempted to concentrate the antitoxin from the milk of immune animals. They tested a large number of precipitating agents, including acetic acid, tannin, oxalic acid, tartaric acid, acetic acid and sodium chloride, acetic acid and ferrocyanide of potassium, alcohol, metal salts, sodium chloride, phosphate of calcium, magnesium hydrate, and aluminium hydrate. Ammonium and magnesium sulphate, etc. were found to be the most suitable. They used the principle of fractional precipitation and found that the addition of 27 % to 30 % of ammonium sulphate gave a precipitate which contained the bulk of the antitoxin, only a trace remaining in the filtrate. In this way they were able to obtain a substance in the form of a powder which was 400 to 600 times as strong in antitoxic power as the original milk.

Brieger and Cohn (1893), employing the milk of animals immunised against tetanus, first clarified the whey by the use of chloroform. They first produced a precipitate with ammonium sulphate, then re-dissolved the precipitate and added basic acetate of lead which throws down a considerable amount of proteid but not the antitoxin. The precipitate resulting from the lead acetate was washed several times in weak alkaline solution, and the filtrates and washings thus obtained were twice treated with ammonium sulphate and the precipitate dried *in vacuo* on clay. The powder was freed from salts by being placed in pure chloroform which allowed the heavier salts to fall and the lighter powder containing the antitoxin to rise to the surface. This concentrated the antitoxin up to 300 or 400 times its original strength. A further purification was attempted by the use of metal salts, alcohol, etc., but without success. On precipitation of the preparation obtained by the use of lead, first with sodium chloride, then with phosphate of soda, and lastly, with ammonium sulphate, the precipitate with the phosphate of soda contained the most antitoxin. It then reached 600 times its original strength.

Wassermann (1894) has improved on the method of concentrating antitoxin from whey. He adds 33 % ammonium sulphate to the whey, filters, and puts the precipitate with a platinum spatula on pieces of clay to absorb moisture. While still damp the precipitate is re-dissolved in one-tenth of the original quantity of fluid. The loss of material is trifling, and practically all the antitoxin is present in the precipitate.

Smirnow (1895) found an antitoxic substance in the magnesium sulphate precipitate from normal, as well as from antitoxic, serum.

Brieger and Boer (1896) succeeded in partly separating diphtheria antitoxin from the adherent albuminous substances by precipitation with neutral salts of the heavy metals. To a fivefold dilution of the diphtheria or tetanus antitoxin double its original volume of a 1 % solution of zinc sulphate or zinc chloride is added. The resulting antitoxin-zinc precipitate is washed with a little water, and dissolved in weak alkaline water (1 : 400 normal alkali). The zinc is precipitated from the solution by the passage of CO_2. If zinc sulphate is used the precipitate contains the antitoxin, but if zinc chloride is used the antitoxin is found in the filtrate. The part containing the antitoxin is dried in the exsiccator to make the zinc-albuminate insoluble, and then the soluble zinc-antitoxin is again dissolved in weak alkali and the remaining zinc again precipitated by CO_2. Brieger and Boer obtained from 10 c.c. of antitoxic serum a deposit of 0·1 gramme which contained all the antitoxin together with albumin, sugar and zinc.

Brodie (1897) precipitated the globulin in four parts by the gradual addition of ammonium sulphate until half saturation was reached. It was found that each of the four precipitates contained antitoxin in almost equal bulk, so that the method did not offer any promise of a possibility of obtaining a stronger solution than could be obtained by re-dissolving the precipitated globulins as a whole. The fact that each of the four fractions contained approximately equal quantities of antitoxin seemed to prove that the globulin precipitate did not carry down the antitoxin by a simple mechanical process. Brodie also stated that antitoxin did not pass through a gelatine filter. These facts appear to indicate that antitoxin is a proteid, belonging, as shown by its solubilities, to the globulin class.

Dieudonné (1897) during a revision of Smirnow's (1895) work failed to find antitoxin amongst the globulins when the precipitation was carried out with carbonic acid. He was able to explain the apparent discrepancy between his results and those of Smirnow by the use of magnesium sulphate, for the globulin thus obtained possessed toxin-neutralising properties. The references already made to the occurrence of antitoxin in the serum of the normal horse (p. 454) make it evident how Smirnow obtained a toxin-neutralising action with the globulin from normal horse serum.

Belfanti and Carbone (1898) could obtain no antitoxin in the precipitate resulting from the use of CO_2 or acetic acid; but succeeded when ammonium sulphate or magnesium sulphate was used.

Szontagh and Wellmann (1898) made a comparison between normal horse serum and antitoxic serum. The specific gravity and the globulin content were the same in both, and in neither was nucleo-albumin present. The antitoxin contained 0·25 % more of total proteids than the normal serum, a fact to which they attached no particular significance. In both cases the chlorine and ash were the same

They made the important observation that in the antitoxic serum there is a lowering of the freezing point and of the electrical conductivity, which appear to be proportional to the amount of antitoxin present.

Freund and Sternberg (1899) tested the action of various chemical agents on antitoxic serum with the view of freeing the antitoxin from the albuminous substances. They obtained the best results by a continued method of precipitation and salting out. They first added potash-alum solution, dialysed the filtrate, removed the resulting slight precipitate by filtration, and by this means got rid of the albumin. The fluid thus obtained was saturated to a half with ammonium sulphate, and the precipitate, which resulted, after being dissolved and dialysed, was concentrated *in vacuo.*

Marcus (1899) showed that there were two forms of globulin in normal serum; the one insoluble, precipitated by carbonic acid and acetic acid, and by dilution with water and dialysis; the other soluble, precipitable by ammonium and magnesium sulphate.

This observation by Marcus proved of great value to Seng (1899) who examined the qualitative and quantitative relations of the proteids in diphtheria antitoxin compared with those in normal horse serum. Seng found that the method first employed by Sternberg of getting rid of a large part of the proteid matter by a potash-alum solution (one-third volume of 5 % solution) was very helpful. This method, which originated from Freund's experiment, was the first to be described by means of which albumin could be got out of an alkaline solution, the globulin remaining in solution. He found that the chief difference between the soluble globulin from normal serum and from antitoxin was that in the case of normal serum there were present precipitation points at 65° C., 68° C., and 71° C.; at 71° C. all the globulin with the exception of traces was coagulated; whereas, in the case of antitoxic serum flocculi first appeared at 71° C., and 75° C. was reached before the bulk was coagulated. Even after this the traces remaining in solution were much greater. From the qualitative examinations no distinct differences could be made out between the soluble globulin of the normal serum and of the antitoxic serum, and the hopes that conclusions might be drawn from the alteration of the quantitative relation of one body in favour of another were not fulfilled. Yet by the exclusion of the albumin and of the insoluble globulin the field for future investigation was narrowed, since it will naturally be limited to the soluble globulin. Whether the differences in the coagulation temperature and in the optical rotation are due to a change in the globulin or to its combination with an antitoxin of unknown chemical composition is a question which must be left open. This work, and the work of Marcus, tend to show that the antitoxins are attached to the globulins which do not go out of solution by dialysis.

Seng's results throw a clearer light on the results previously obtained by Belfanti and Carbone, and Dieudonné. These soluble globulins correspond to the pseudoglobulin of Pick, to which reference will be made later.

Astros and Rietsch (1900) dilute the antitoxic serum fivefold and add enough sodium and potassium chloride to the diluted serum to bring the concentration of these salts up to 20 %. The mixture, after the addition of 0·5 % phenol, is kept at 33° C. for 24 hours. The antitoxin is thus thrown out of solution together with the globulins.

Atkinson (1899—1900) investigated the fractional precipitation of the globulin and albumin of normal horse serum and of diphtheria antitoxic serum, and tested the antitoxic strength of the various precipitates. He found that the globulins from both normal and anti-diphtheritic sera react in the same way towards reagents. All the antitoxic power of normal and immune serum is carried by the globulin and its fractional precipitates, a certain loss of globulin and antitoxic power occurring during the manipulations. He believes, from the reactions obtained and from the fact that diphtheria antitoxin is contained in normal horse serum and that this natural antitoxin separates with the globulin, that the diphtheria antitoxin is probably a kind of globulin.

Hiss and Atkinson (1900—1901) continued these studies. By the use of magnesium sulphate they obtained a precipitate of globulin which appeared to contain all the antitoxin in the serum, for the precipitate from 1 c.c. of serum protected guinea-pigs against the same amount of toxin as 1 c.c. of the serum. They made the interesting observation that the globulin precipitate is increased as the result of immunisation, for they found that antitoxic sera on the average gave a higher globulin precipitate than did normal sera, and that the serum of the same horse after immunisation gave a larger precipitate of globulin than before immunisation. They conclude that antitoxin formation is probably accompanied by new globulin formation.

Pick (1902) found that on the addition of 21·5 % of ammonium sulphate to anti-diphtheritic serum a precipitate formed, but no antitoxin was present in it = Fraction I.

When the ammonium sulphate reached a concentration of 25·6 % a second precipitation of globulin occurred which still contained no antitoxin = Fraction II.

A fraction of proteid precipitated by the addition of ammonium sulphate up to 38 % contained practically the whole of the antitoxin = Fraction III.

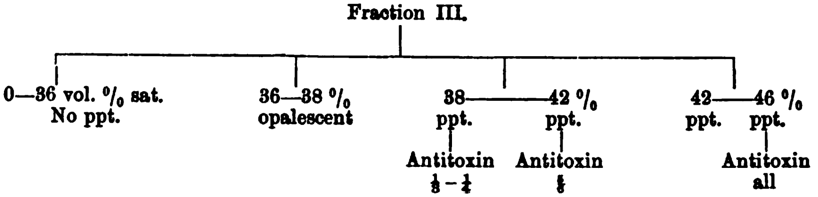
In solution there remained only the serum albumin = Fraction IV.

Pick states that Ide and Lemaire (1899) obtained similar results.

The relations of these fractions to the substances called by Hofmeister "Euglobulin" and "Pseudo-globulin" and their fractional precipitation points with ammonium sulphate, as found by Fuld and Spiro (1900), are shown in the following Table:—

Fibrinoglobulin	Euglobulin	Pseudo-globulin	Albumin
Fuld and Spiro.			
0—21·5 vol. % sat.	28—36 vol. % sat.	36—44 vol. % sat.	100 vol. %
Pick. Fraction I.	Fraction II.	Fraction III.	Fraction IV.
0—21·5 % $AmSO_4$	21·5—25·6 %	25·6—38 %	38—76 %
$0—\frac{1}{3·5}$ sat.	$\frac{1}{3·5}—\frac{1}{3}$ sat.	$\frac{1}{3}—\frac{1}{2}$ sat.	$\frac{1}{2}$ to sat.
0—28·3 vol. % sat.	28·3—33 vol. % sat.	33·3—50 % sat.	50—100 vol. % sat.

Fraction III when re-dissolved and re-precipitated gave the following result.



The filtrate containing only the albumin had no antitoxic value. The above result was obtained with horse antitoxin. The interesting observation was made that in the case of goat's serum the antitoxin came down, not with the fraction corresponding to the pseudo-globulin, but with the fraction corresponding to the euglobulin. It will be remembered that by Brieger and Ehrlich's method of precipitating antitoxin from the whey of the goat a great loss of antitoxin was encountered during the dialysis. The loss can be explained in the light of the interesting observation made by Pick, that the antitoxin in the case of the goat goes down with the euglobulin, for we know that the euglobulin in salt solution goes out of solution on dialysis.

Pröscher (1902) claims to have obtained by trypsin digestion an antitoxin which gives no proteid reactions. This observation, if confirmed, would be of the greatest interest and importance on both theoretical and practical grounds.

Gibson (1905—1906) precipitates the serum with an equal volume of saturated ammonium sulphate solution, filters, and extracts the residue with a saturated solution of sodium chloride. The globulin containing the antitoxin, the pseudo-globulin, is soluble in the sodium chloride solution, whereas the globulin not containing antitoxin, the euglobulin, the proteids, etc., are precipitated and allowed to sediment. After filtration the sodium chloride solution of antitoxin is precipitated by the addition of a half volume of saturated ammonium sulphate or with acetic acid. The filtered precipitate is pressed dry with paper and dialysed in parchment. A double filtration through Berkefeld filters is employed for sterilisation. One quarter to one half per cent. sodium chloride and a preservative are added. After being tested for potency, sterility, etc. this antitoxin is employed in practice.

By this method a serum, satisfactory in regard to appearance, absence of high viscosity, etc., and of at least twice the original antitoxic potency may be comparatively easily secured.

In a horse which yielded high-grade antitoxin Ledingham (1907) showed that the total globulin progressively increased during immunisation to double its initial value. In a refractory horse which failed to yield high-grade antitoxin no such progressive rise was observed.

Pick's statement, that the antitoxin in the case of the goat is precipitated with the euglobulin and not with the pseudo-globulin as in the horse, has not been confirmed either by Gibson and Collins (1907) or by Ledingham (1907).

Banzhaf and Gibson (1907) found that the serum globulins soluble in saturated sodium chloride solution of the higher fractions are uniformly much more potent in antitoxin per gram of proteid, than are those precipitated by lower concentrations of ammonium sulphate.

THE MECHANISM OF ANTITOXIN PRODUCTION.

Antitoxin was at first regarded as a direct derivative of toxin which by some process taking place in the animal body became converted into antitoxin, in other words, that the antitoxin was a modified toxin. This hypothesis, which was suggested by Buchner (1893), seemed to be a natural deduction from the facts as known at the time. An animal injected with a toxin developed antitoxin in its blood; it seemed, therefore, probable that the animal body had acted on and modified the toxin in such a manner as to convert it into antitoxin. A more intimate investigation of the question raised many difficulties in the way of accepting this view. Exact determinations showed an absence of quantitative relation between the amount of toxin injected and the amount of antitoxin produced in the animal body. Knorr (1899) showed that the injection into an animal of an amount of toxin which could be neutralised by one unit was followed by the production of 100,000 units. Ritchie (1901) in immunising guinea-pigs with tetanus toxin found that twice the amount of toxin gave a serum one hundred times stronger. It has been suggested that the toxin molecule might split up into molecules of smaller size, each containing a group capable of neutralising a toxin molecule. We have reason to believe however, from the work of Brodie, Martin and Cherry, etc., that the antitoxin molecule is probably larger than the toxin molecule, consequently it is difficult to understand how the smaller toxin molecule could be so modified in the animal body as to give rise to a much greater number of larger antitoxin molecules. There is also no evidence in favour of the splitting up of the toxin molecule into parts which go towards the constitution of the antitoxin molecule. A very strong argument against the view that the toxin is directly employed in the building up of the antitoxin was furnished by Roux and Vaillard (1893), who found that in the case of a rabbit immunised against tetanus quantities of blood equal to the total volume contained in the animal's body could be removed within a few days, and yet no diminution in the antitoxic value of the blood could be determined. Similar results were obtained by Salomonsen and Madsen and by the author in the case of goats and horses immunised against diphtheria. The loss of antitoxin which occurs immediately after the removal of blood is quickly made good by a supply which must be derived from the cells and tissues of the body. As this occurs without the re-injection of toxin it is improbable that the latter can in any way be concerned in building up the new antitoxin. These considerations lend increasing support to the view that the animal body furnishes the antitoxin by a process analogous to secretion.

Ehrlich's Theory of the Mechanism of Antitoxin Production.

Ehrlich holds that the fundamental processes of antitoxin production reside in the cells for which the toxin has a selective affinity. The cells may be those of any organ or tissue, but it is essential that they should be capable of being affected by the toxin. The cell protoplasm, which must be regarded entirely from the chemical standpoint, consists of living molecules of great complexity. These molecules have a central nucleus with lateral or side-chains which are capable of binding the molecule to other molecules having the proper affinities. Each molecule of protoplasm has many different kinds of side-chains which have affinities for different substances (see Fig. 3). These side-chains are the means by which the nutrition of the cell is maintained, their normal function being to attach to themselves certain food stuffs. These food stuffs must have their atom groups so arranged that they fit the

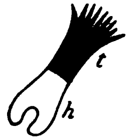


Fig. 1.

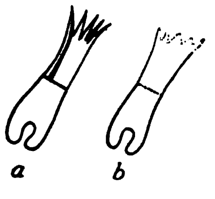


Fig. 2.

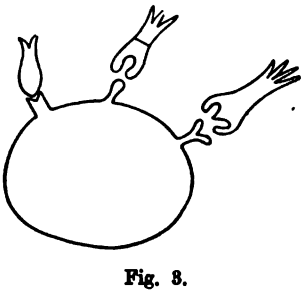


Fig. 3.

Fig. 1. Toxin molecule. *h* = haptophorous group. *t* = toxophorous group.

Fig. 2. Toxoid molecule with (*a*) a trace only (black), (*b*) none of the toxophorous group left.

Fig. 3. Diagram showing various forms of receptors or side-chains and various substances having the proper relation of outline to these receptors.

corresponding side-chains or receptors of the cell molecules. The side-chains of the food must have a specific relation to the side-chains of the cells, or as Emil Fischer has expressed it they must be adapted to one another as the key to the lock. The receptors, in addition to their normal function of assisting in the nutrition of the cells, possess fortuitously and incidentally the capacity of anchoring to themselves this or that toxin. One must here revert to Ehrlich's view (see p. 475) that the diphtheria toxin molecule consists of two groups of atoms (see Fig. 1), the toxophorous group (*t*), to which the poisonous properties of the toxin are due, and the haptophorous (ἅπτω = I seize) group (*h*), by means of which the connection between the cell or the antitoxin and the toxophorous molecule is established. It is by means of these haptophorous groups then that the toxin molecules become attached to the "side-chains" or receptors of the cells of the animal body (see Fig. 3). The haptophorous group of one toxin finds the suitable receptors in one kind of cell, the haptophorous group of another toxin in another kind of cell, and in this way we have an explanation of the specific action of toxins.

The first stage of toxin action is the anchoring of the toxin molecule by its haptophorous group on to the suitable receptor of a certain cell (see Fig. 4), and this likewise is the first stage in antitoxin production. The receptor while it is occupied in this manner cannot exercise its normal physiological function of conveying nutrition to the cell protoplasm. Thus there is created a defect in the cell-life which the cell endeavours to make good by a process of regeneration. Fresh side-chains are produced (see Fig. 5), and as these are occupied again and again by fresh injections of toxin the attempt at compensation gives rise to the production of an excess of side-chains in accordance with the principles enunciated by Weigert. The side-chains then being produced in excess of the requirements of the cell are thrown off into the blood stream where they constitute the antitoxin (see Fig. 6). In their free state in the blood stream they are still capable of union with the toxin, which is thus diverted from attacking the susceptible cells (see Fig. 7).

It follows from Ehrlich's view that the toxoid having the same combining power for antitoxin as the original toxin should also be capable of occupying the corresponding side-chains of the cells in the animal body, and should thus produce the necessary condition for the stimulation of the cell to the hyper-production of side-chains, that is, to antitoxin production. Ehrlich assumes throughout most of his work

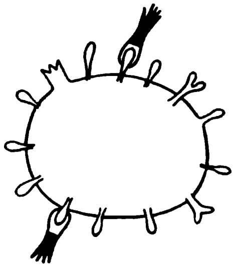


Fig. 4.

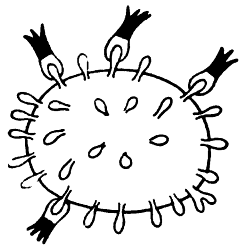


Fig. 5.

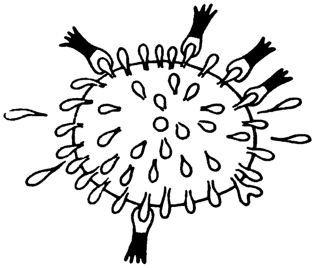


Fig. 6.

Fig. 4. First stage of antitoxin formation; toxin molecules anchored on to the suitable receptors.

Fig. 5. Second stage of antitoxin production. To compensate for the number of receptors thrown out of action owing to their being occupied by toxin molecules there occurs a production of side-chains in excess of the normal.

Fig. 6. Third stage. Hyper-compensation takes place so that a larger number of receptors is provided than is necessary for the cell life. These unnecessary side-chains are thrown off into the blood and constitute the antitoxin.

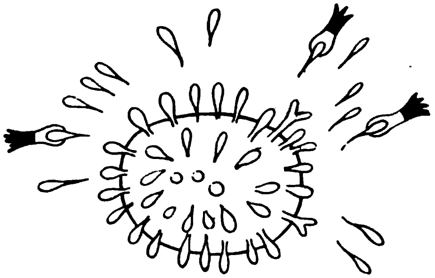


Fig. 7. Showing combination of free receptors, or antitoxin, with toxin molecules in the blood, and demonstrating the mechanism of cell protection by means of antitoxin.

that toxoids are capable of giving rise to the production of antitoxin.

It will be recalled that several of the methods suggested for the early stages of immunisation are based on the view that toxins modified by heating and chemical agents are still capable of stimulating the animal body to produce antitoxin. The question has been raised as to whether the mere occupation of the receptors is sufficient to stimulate the cell to antibody production. Von Dungern (1902) was the first to suggest that something of the nature of an irritative stimulus was necessary in addition to the occupation of the receptors. His view was largely based on the fact that during the ordinary nutritive processes nothing of the nature of an antibody or precipitin could be found in the blood. Ehrlich, Morgenroth (1902), and Wassermann (Brussels Congress, 1903) have, therefore, assumed that in addition to the occupation of the receptors there must be a stimulation or irritation of the cell, which is probably to be attributed in the case of toxic substances to traces of the toxophorous groups which have remained undestroyed. Bruck (1904) has further investigated this point in relation to tetanus toxin. He finds that tetanus toxoid, though it can combine with tetanus antitoxin, is not by itself capable of giving rise to the formation of antitoxin, but that if a mere trace of toxin is present antitoxin is produced. The mere occupation of the receptors by the haptophorous group is in this case not sufficient for antitoxin formation; the toxophorous group must be present to give the necessary stimulus to the cell.

The question as to whether diphtheria toxones can produce antibodies, and the nature of the latter (if any) is of great theoretical interest. The point was investigated by Madsen and Dreyer who found that toxones were capable of actively immunising the guinea-pig, rabbit, goat and horse, and that the serum in all cases, except in the rabbit, contained antitoxin. The toxones, therefore, have haptophorous groups which are closely allied to or identical with those of the toxins[1].

A remarkable confirmation of Ehrlich's theory was afforded by Wassermann (1898), who argued that, if Ehrlich's hypothesis was true, antitoxin in its pre-formed state ought to be capable of demonstration in the normal susceptible cells of certain organs, since certain toxins attack them in a specific manner. For example, we know that tetanus toxin attacks the cells of the nervous system, therefore in these cells antitoxin ought to be demonstrable. On applying the test of experiment to this view he was able to show that an emulsion of guinea-pigs' brain could fix the tetanus toxin and neutralise its action[2]. Roux (1898) argued that if the nervous system contained antitoxin toxin injected into it should be at once neutralised, and cause no toxic effects. He demonstrated, however, that the intracerebral injection of tetanus toxin causes the death of the animal, and that smaller quantities are necessary to produce a lethal effect by this method than by the ordinary methods of injection. He held that the result was not compatible with Ehrlich's theory. Ehrlich, however, has pointed out that Roux's results so far from being in opposition to his theory are confirmatory of it. The receptors, so long as they are attached to the cells, are the connecting links between the cells and the toxin, and are thus in a sense a danger to the cells, whereas, when they are thrown free into the blood stream, still possessing as they do the property of attracting the toxin molecules, they form a protection to the cells from which they divert the toxin. Weigert illustrates this relation by a reference to the action of a lightning conductor. The copper or iron rod if placed inside the building without a suitable earth connection would be an added danger, whereas, when insulated and separated from the building, it affords protection by conducting the electricity to earth.

---

[1] Madsen's later view will be dealt with elsewhere.

[2] The interpretation of this experiment has led to much controversy, into the discussion of which it would be impossible to enter here.

CHAPTER XVII.

THE EFFECTS OF ANTITOXIN ON TOXIN.

Theories regarding the interaction between antitoxin and toxin. Further analysis of the method of action of the antitoxin on the toxin. Brief summary of Ehrlich's theory of the relations of antitoxin to toxin. The physico-chemical theory of Arrhenius and Madsen. Criticisms of the views of Arrhenius and Madsen. The adsorption views of Bordet. The behaviour of antitoxin in the animal body when used for the production of passive immunity. The disappearance of antitoxin from the blood. Absorption of antitoxin from the alimentary canal. The question of the production of anti-antitoxins. Inherited immunity. Transmission of inherited immunity. Antimicrobial diphtheria serum.

Theories regarding the Interaction between Toxin and Antitoxin.

Soon after the discovery of antitoxin the nature of the interaction between toxin and antitoxin became a subject of investigation and controversy.

Behring held that the antagonism was of a chemical nature, and that the antitoxin neutralised the toxin in the same way as an alkali neutralises an acid. On the other hand, Buchner maintained that the antitoxin did not act directly on the toxin but indirectly through the intervention of the cells. The controversy on the subject between Behring and Buchner, which took place in 1894, was soon followed by a series of investigations on the subject. The chief supporters of the chemical view were Behring and Ehrlich, the opposition in favour of the cellular and vital hypothesis was led by Buchner, Metchnikoff, Roux, and Calmette. The death-blow to the cellular hypothesis was given by Ehrlich, who by a beautiful experiment was able to demonstrate the neutralising action of antitoxin on toxin *in vitro* in the absence of all living cells.

Ricin, when brought into contact with a suspension of dead red blood corpuscles in a test tube, causes their agglutination; when, however, a suitable quantity of antitoxin is added, the agglutination no longer takes place. Ehrlich was further able to show that when antiricin was added to a solution of ricin, in sufficient quantity to inhibit its agglutinating power on red blood cells, its toxic action on animals was also abolished. He thus proved in this case that the neutralisation which could be demonstrated *in vitro* was valid *in corpore.*

Since Ehrlich's fundamental experiment a large number of similar test-tube experiments have become possible with the expansion of the knowledge of haemolytic and agglutinating agents of animal, vegetable and bacterial origin: we need only mention cobra venin (Stephens and Myers), abrin (Ehrlich), tetanolysin (Ehrlich and Madsen) as examples of a large group of similar substances which produce haemolysis or agglutination of red blood corpuscles.

The experiments carried out by Morgenroth on rennin, Dean and Achalme on trypsin, and Sachs on pepsin with their corresponding antibodies have satisfactorily demonstrated the neutralisation of toxin by antitoxin (ferment by anti-ferment) in the absence of all cellular elements.

In this connection also may be mentioned the precipitin experiments of Kraus, Tchistowitch and Bordet. These experiments afford conclusive proof that the action of the antitoxin on the toxin is direct and not through the intervention of the cells of the animal body.

Further analysis of the method of action of the antitoxin on the toxin.

Does the antitoxin combine with the toxin so as to convert it into an atoxic modification thus causing its destruction as toxin, or does it simply neutralise the toxin forming an atoxic combination by a process analogous to the neutralisation of an acid by a base with the resulting formation of a neutral salt? Calmette (1895) found that whereas cobra venin is not affected antivenin is destroyed by heating to 68° C. for 10 minutes. Mixtures in certain proportions of venin and antivenin produced no symptoms when injected into rabbits of a certain weight. Similar mixtures after standing 10 minutes, and thereafter being heated to 68° C. for 10 minutes before injection into rabbits of the same weight, rapidly caused their death.

Wassermann (1896) found that *pyocyaneus* toxin was not affected by temperatures which completely destroyed its antitoxin. By heating newly made neutral mixtures he was able to demonstrate that the toxin, uninfluenced by its association with the antitoxin, was still capable of killing animals. Both Calmette and Wassermann arguing from these experiments gave their support to the vital theory. Marenghi (1897) carried out analogous experiments with diphtheria toxin and antitoxin mixtures. In this case, however, it is the toxin which is destroyed by heating, the antitoxin remaining unaffected. It had been shown by Roux and Yersin that diphtheria toxin is destroyed at 60° C., whereas antitoxin is unaffected by temperatures up to 70° C. Mixtures of toxin and antitoxin, which were neutral for the guinea-pig, after being heated to temperatures sufficient to destroy the toxin, were demonstrated to contain free antitoxin since additions of fresh toxin were found to be neutralised.

Martin and Cherry (1898) pointed out that previous investigators had in their researches neglected the *time* factor, which may be a very important element in any possible chemical interaction between toxins and antitoxins. The experiments, for example, of Calmette and Wassermann can be repeated and their results confirmed, and yet by modifications of the factors, *time*, *temperature* and *active masses*, exactly opposite results may be arrived at, proving that their conclusions are unjustified. Martin and Cherry in their series of experiments on the diphtheria toxin and antitoxin combination employed the gelatin filter of Martin, which consists of a Pasteur-Chamberland filter impregnated with gelatin. Through such a filter a diphtheria toxin can be forced by a pressure of 50 atmospheres without undergoing any very marked loss in toxicity, whereas, as shown by Brodie, antitoxin owing to the fact that it possesses a larger molecule cannot pass through, but is held back along with the proteids. Neutral mixtures of diphtheria toxin and antitoxin, which had been allowed to remain in contact at 30° C. for two hours, were filtered through the gelatin filter. The filtrate was found to be innocuous to guinea-pigs even in quantities corresponding to 32 fatal doses. From this experiment the deduction is made that the smaller molecule of toxin must have entered into some sort of chemical relationship with the relatively large molecules of antitoxin, during their sojourn together, prior to filtration.

The value of the factors, time and influence of proportion of active masses, was shown by a series of experiments on the snake venin of *Hoplocephalus curtus*, and its antivenin. The mixtures of two, three and four lethal doses of venin with 1 c.c. of antivenin were allowed to stand at 20—23° C., for varying times, 2, 5, 10, 15, and 30 minutes, and then injected into rabbits. The results are shown in the following table:

Proportion of toxin to antitoxin per kilo. of body wt.		Control, venom only	Time allowed for interaction of toxin and antitoxin: temp. 20—23° C.					
Antitoxin	Toxin		2 mins.	5 mins.	10 mins.	15 mins.	30 mins.	Injected unheated ∞ mins.
1 c.c.	2 fatal doses	Died 15 hours	Lived (very ill) for 2 days	Lived (ill 1 day)	Lived (no symptoms)	Lived (no symptoms)	Lived (no symptoms)	Lived (no symptoms)
1 c.c.	3 fatal doses	Died 12 hours	Died 20 hours	Died 28 hours	Lived (ill 2 days)	Lived (ill 1 day)	Lived (no symptoms)	Lived (no symptoms)
1 c.c.	4 fatal doses	Died 9 hours	Died 13 hours	Died 15 hours	Died 23 hours	Lived (very ill 2 days)	Lived (no symptoms)	Lived (no symptoms)

In this connection Danysz' (1902) experiments on the action of gastric juice on neutral ricin-antiricin mixtures are of great interest. When ricin and antiricin are submitted to the action of gastric juice for 16 hours at 40° C., the antiricin is found to have been completely destroyed, whereas the ricin is only slightly attenuated. A mixture of ricin and antiricin is prepared so that it has no agglutinating action on the red blood corpuscles as tested *in vitro* and no toxic action as tested on guinea-pigs. When this neutral mixture has been digested in the manner indicated it is found that the proteolytic ferment, while destroying the antiricin, leaves the ricin but little influenced so that the mixture becomes agglutinating and toxic.

These experiments, while proving that something of the nature of a chemical union takes place between the toxin and antitoxin, clearly show that the antitoxin does not destroy the toxin, since it is possible to recover it unaltered from neutral mixtures.

While the majority of investigators now adhere to this view of a direct action of antitoxin on toxin, there is still great divergence of opinion as to the exact mechanism of the interaction, and after this preliminary statement the more important views will be dealt with under separate headings.

BRIEF SUMMARY OF EHRLICH'S THEORY OF THE RELATIONS OF TOXIN TO ANTITOXIN, AND ON THE MECHANISM OF THE PRODUCTION OF ANTITOXIN.

Ehrlich's theory, owing to the important influence it has had in the investigation of the whole subject of diphtheria immunity, has already been dealt with in various sections of this work. Notwithstanding that it entails a certain amount of repetition, it has, however, been considered desirable to give here a brief summary of the subject so that the argument may be presented as a whole.

Judging from the history of the ferments, whose close relationship to the toxins was early pointed out by Roux, we cannot hope for much help from the purely chemical side in elucidating the problems connected with toxins. At the same time the most intimate knowledge that we possess of the nature of toxins has been obtained by the use of physico-chemical methods. Ehrlich obtained many of the most important facts on which he bases his views of the constitution of diphtheria toxin by using the method of partial saturation of the toxin with the antitoxin. If a mixture of hydrochloric and acetic acids is gradually saturated with an alkali the bulk of this goes first to the acid of stronger avidity, the hydrochloric acid, and then afterwards to the weaker acetic acid. Ehrlich has found analogous facts when diphtheria toxin is partially saturated with antitoxin, and by graphic demonstration of the course of the neutralisation has shown that the constitution of diphtheria toxin is much more complex than was at first surmised, and that the toxin and its derivatives possess quite different affinities for the antitoxin. Similar results were obtained by a number of workers in regard to other toxins. Ehrlich was also influenced in the elaboration of his views by those of Emil Fischer on the atom groupings of ferments. Fischer found that only such substances as possess a group into which the ferment group fits as the key into the lock are acted on by a particular ferment, and that it is only through the relation of these two groups, the one belonging to the ferment, the other to the fermentable substance, that there is obtained the union of the two molecules which is essential for the chemical action.

Ehrlich, applying these views to the present question, holds that the specific relationship of toxin to antitoxin can be most simply expressed by the assumption of two groups which correspond in their configuration. He assumes that the toxin molecule contains two functionating groups: a haptophorous, and a toxophorous, group. In favour of the assumption of a toxophorous group was the wide experience which had been obtained of the relationship between chemical constitution and physiological action. Thus, it had been shown that certain physiological actions can only be ascribed to certain groups, for example, in a great number of hypnotics only the aethyl group, and in a great number of anaesthetics only the benzoic acid radicles are concerned. It was a natural inference, therefore, to assume in the case of the toxins a special group giving rise to the toxic condition, that is, a toxophorous group.

As a strictly chemical characterisation of the toxins is not possible the criteria of the toxin nature must be purely biological. Especially marked are the nature of the toxic action and the ability to produce specific antibodies in the animal organism. The toxins share this property with a number of other substances; some of them, like the ferments, more or less toxic, others, as the albuminous and cellular substances, quite harmless. The period of incubation following toxin administration cannot, in most cases, be curtailed beyond a certain time, however much the dose is increased. The latent period in most instances lasts from six to twenty-four hours but may extend, as in diphtheria, to 14—20 days. It is doubtful if latency is an essential characteristic of toxin action, since the closely allied venin, ichthyotoxin and haemotoxin can act without any incubation period. A much more definite characteristic is the capacity to furnish antitoxin, since this is not shared by substances possessing a well-defined chemical composition. Ehrlich failed to produce antisera for alkaloids and toxic glucosides, and also failed to confirm the view that tetanus antiserum has an antitoxic action against strychnia.

In his earlier work Ehrlich showed that such substances as the alkaloids and colouring materials are present in the tissues, not in the form of close chemical combinations, but only loosely anchored to the cells. Corresponding to the loose character of the combination is the short duration of the action of such bodies as antipyretics, compared to the long lasting injuries which the toxins produce, and which point to their having taken a firm hold on the protoplasm. Those substances which form a lasting combination with protoplasm and enter into its constitution have long been stated to be "capable of assimilation," and most of these bodies have been held to belong to the group of nutrient substances. Ehrlich holds that this capacity for assimilation must depend on a chemical combination. He assumes that for such union there must be present two atom groups which have an attraction for one another, and he, therefore, suggests that the protoplasm molecule must have side-chains or receptors which have a maximum chemical affinity for certain groups of the specific nutrient substances which are thus anchored to the cells. To make the essence of toxin action comprehensible is, as Ehrlich asserts, only a short step. It is enough to assume that the toxins which, in their origin and in their chemical properties, may be regarded as closely allied to the albumins, peptones, etc., also possess groups which correspond to those of the nutrient substances, and which have the capacity to combine with the receptors of certain cells. Such atom groups of the toxin molecule Ehrlich calls "haptophorous groups" because they produce the fixation on to the cells. This view has been strengthened by numerous observations which prove that quite a number of substances, which are not toxins but nutrient substances, produce in the animal organism genuine antibodies. Bordet, for instance, showed that milk, various forms of serum, etc., also produce antibodies, "precipitins," which precipitate the corresponding body in a specific manner. The chief evidence in favour of the two functions, the haptophorous and the toxophorous, is based largely on observations on hemitoxin, in which half of a toxin becomes converted into an atoxic substance or toxoid which has still the power of combining with the same amount of antitoxin. The easiest explanation of the phenomenon is that the toxoids possess haptophorous but not toxophorous groups.

With the help then of the haptophorous group the toxin molecule is anchored on to the cell. This, however, does not sufficiently explain the toxic action. The presence of the second already mentioned toxophorous group is necessary. It acts on the protoplasm by means of the intermediate action of the haptophorous group.

On such an hypothesis Morgenroth explains in the simplest manner the interesting observation, first made by Courmont, that frogs injected with tetanus toxin if kept in a cool place do not develop tetanus, but if placed in an incubator at 37° C., quickly become tetanic and die. That the toxin was already fixed to the cell was proved by injecting sufficient antitoxin to neutralise the toxin, and after sufficient time for union had elapsed placing the frogs in the incubator. In spite of the presence of the antitoxin they developed a fatal tetanus proving that the tetanus toxin had become fixed to the cells in sufficient quantity to cause a fatal tetanus.

Ehrlich also instances the fact, observed by Billinger and H. Meyer, that marmots and bats are not affected by tetanus toxin during their winter sleep, but succumb to its action on awaking. Both these interesting and anomalous observations may be explained on the hypothesis of two groups being present, the haptophorous groups being capable of becoming fixed on to the nerve cells at a lower temperature than is required for the action of the toxophorous groups.

The incubation period can be explained easily in this manner. The haptophorous group quickly combines with the cell, the toxophorous group comes more slowly into action. This is confirmed by the experiments of Dönitz and Heymans who showed that when tetanus or diphtheria toxin is injected into an animal it disappears very quickly from the blood, and must, therefore, be fixed to the tissues and organs. This disappearance from the blood occurs long before the onset of any signs of intoxication. That the toxin has become fixed on to the susceptible tissues is proved by the method of injecting antitoxins at various periods after the toxin, and ascertaining the interval of time after which the antitoxin is no longer capable of saving the life of the animal. The experiment was so arranged that when a certain quantity of serum was injected at the same time as the toxin its action was completely neutralised. After the lapse of six minutes the corresponding dose of serum was no longer capable of saving the animal's life, showing that some of the toxin had already become fixed on to the susceptible cells. Yet in such a case no symptoms would appear till 12 to 24 hours after the toxin injection. This is strong evidence in favour of the haptophorous group having become fixed on to the suitable side-chain long before the toxophorous group came into action. In the above experiment if multiples of the quantity of serum were employed the animal could still be saved, showing that the cells, to a certain extent, were capable of being freed from the toxin already anchored on to them[1].

The susceptibility of the organism to poisons depends, therefore, on the presence of suitable "receptors." Many forms of natural immunity and certain forms of acquired immunity may be ascribed to the fact that suitable receptors are absent or defective. That poisons such as diphtheria toxin only harm certain species of animals, such as mice, in very large doses, whereas others, such as guinea-pigs and rabbits, succumb to the minutest dose may be easily explained by assuming that the cells of the insusceptible animals do not possess the suitable receptors. If the receptors which have a particular affinity for a toxin are distributed widely through the tissues or organs of an animal's body, the dose of toxin necessary to kill such an animal will be greater than in the case of an animal in which the receptors are confined to one organ of great importance to life such as the brain.

---

[1] H. Meyer's experiments showing that tetanus toxin is conveyed along the nerve trunks may require some modification of these views.

In the one case the toxin will have its activity diffused and will be diverted from important organs; in the other case, the toxic force will be concentrated on cells, an interference with whose action endangers the life of the animal. The large swellings in the connective tissue which diphtheria toxin, ricin, and abrin produce can only be explained by an anchoring of the toxin on to the subcutaneous connective tissue cells. In this connection it is interesting to note that the delicate connective tissue of the mouse is untouched by diphtheria toxin, whereas a hundredth or thousandth part of the dose produces in the guinea-pig an extensive necrosis of the skin. We may here recall Buchner's experiment wherein he observed that a mixture of tetanus toxin and antitoxin, which was harmless to the mouse, was still capable of causing tetanus in the guinea-pig. The probable explanation is that a certain amount of toxin remained unneutralised in the mixture, and Ehrlich suggests that in the body of the mouse there exist a number of receptors distributed through the body, whereas in the guinea-pig most of the receptors are confined to the nerve cells. This may also explain the fact that the guinea-pig is much more susceptible than the mouse. In this way it is easy to understand how the trace of unneutralised toxin, finding its way direct to the important nerve cells of the guinea-pig, manifests its toxic action.

Receptors in Production of Antitoxin.

If the receptors of a cell are occupied by toxin a defect in the cell life is produced and the affected side-chain can no longer exercise its normal function of taking up nutrient substances. According to Weigert's law this defect will not only be compensated but over-compensated. This over-compensation can, through continued injections of appropriate amounts, be carried so far that the receptors are produced in such abundance as to be no longer of value to the cells, and are thrown as useless ballast into the blood fluid. These thrown-off receptors, or side-chains, according to Ehrlich's theory, constitute the antitoxin, which when present in the blood stream can, by uniting with it, divert the toxin from the susceptible organs.

This theory explains the multiplicity of the antitoxins and their specific character on the basis of normal physiological processes. Corresponding to their origin the relation of the antitoxins to the toxins is of a purely chemical nature; by their union they form a new combination indifferent to the animal body as has been shown by Fraser, Ehrlich, Cherry and Martin, Behring and Knorr.

The Constitution of the Toxin.

Ehrlich became convinced that the toxins were more complex in their nature than had been generally assumed. When we consider what a large number of substances one cell can produce (the bark of the quinine plant alone produces about 20 different alkaloids, quinine, cinchona, cinchonidin, etc.), it seems improbable *à priori* that a bacterial cell should produce a single poison. We must in future reckon with the probability that the products of secretion of bacilli contain quite different forms of poison. Ehrlich, for example, showed that tetanus filtrates contain, in addition to the spasm producing poison, one which dissolves the red blood corpuscles. Through Ehrlich's, and later Madsen's, researches it was shown that tetano-spasmin and tetanolysin possess different haptophorous groups which fit different receptors. The two corresponding antibodies are produced together but in different proportions during immunisation.

A further question which arises is whether secretion products of bacteria which possess similar haptophorous groups are of identical nature. The investigation of diphtheria toxin convinced Ehrlich that this is not so, but that toxic varieties exist to which a common haptophorous group is suitable. Besides the typical toxin the diphtheria bacillus produces at the same time and commonly in like quantity a second product, "toxone," which is strongly differentiated from the chief poison by its action on the animal body. Through the researches of Madsen, Ehrlich, Woodhead and others we know that diphtheritic paralysis, the onset of which takes place long after the acute intoxication caused by the toxin has disappeared, is due to the action of the toxone. The toxone is also characterised by the fact that its local action is slighter than that of the toxin. It produces no necrosis but only slight transitory inflammation, which may be absent in the case of small doses.

It must be assumed that the toxone possesses the same haptophorous group as the toxin, since it is completely saturated by antitoxin. The relationship of the toxone-haptophorous group to the antitoxin is distinctly weaker than that of the toxin. If a mixture of both substances is partially saturated with antitoxin the toxins are first neutralised and later the toxones in a manner similar to the neutralisation of a mixture of hydrochloric acid and acetic acid by a base, in which case the hydrochloric acid is first saturated before any combination occurs with the acetic acid. The functional difference of the two bodies is probably due to the fact that the nature of their respective toxophorous groups is essentially different.

The toxones are not products derived from the toxins since in both experimental and clinical observations, where the living bacilli are concerned, toxone action is clearly to be seen, and, since we believe that the toxic products which are produced in the body are at once absorbed without having time to pass into modified forms, we must assume a primary production of the toxones, in favour of which assumption other purely clinical observations may be adduced.

Of greater importance are certain modifications of the toxin, namely toxoids. If the same amount of antitoxin, for example the "immunity unit," saturates in the case of one toxin a quantity which can kill 100 guinea-pigs, and in a second only the lethal dose for 15 guinea-pigs, it must be assumed that in the toxin broth other substances are present in addition to the toxin which have a specific affinity for the antitoxin. Prolonged examination of the loss of toxicity of a toxin gave accurate information in regard to the meaning of these substances. It was found that during the spontaneous weakening of a toxin the neutralisation constants L_0 and L_+ can remain completely unchanged, while the "absolute toxicity" goes down to a half or even lower. It follows from this that the toxophorous group only, and not the haptophorous group, has been damaged. We must, therefore, assume that the toxophorous complex is more easily affected by chemical or thermal injuries than the haptophorous, and that "toxoids," the substances which are non-toxic but are nevertheless capable of uniting with antitoxin, occur as the result of the changes produced by such influences.

It is probable that thermal and chemical agents, though capable of producing completely atoxic substances which retain the power of immunising animals, do not affect a substance of such complex structure as the toxophorous group uniformly, but may act in a graduated fashion leaving traces of toxic substances which may be demonstrated only on particular races of animals.

Toxoids and Toxones as Immunising Agents.

That toxoids can give rise to antitoxins is held by Ehrlich to be a strong support to his view that two groups, the haptophorous and the toxophorous, are present in the toxin. He holds, for example, that animals can be immunised against tetanus by the use of toxoids, and that the only easy explanation is the assumption that the haptophorous group has remained intact and capable of occupying the side-chains of the antitoxin-producing cells, which are thus stimulated to elaborate and throw off fresh side-chains, in other words to produce antitoxin.

THE PHYSICO-CHEMICAL THEORY OF ARRHENIUS AND MADSEN[1].

Arrhenius and Madsen have recently brought forward another theory of the constitution of toxin and of the nature of the combination which takes place between toxin and antitoxin. They hold that the assumption of such a complex structure for diphtheria toxin as postulated by Ehrlich is unnecessary. They base their views on a number of observations obtained by using physico-chemical methods of investigation: in reality, by an extension of the methods of investigation which were first introduced into the subject with such conspicuous ability by Ehrlich.

Ehrlich, as we have seen, came to the conclusion that the union between toxin and antitoxin was similar in nature to the union between a strong acid and a strong base. Starting his argument from that point he arrived at the conclusion that diphtheria toxin was built up of a number of substances of greater or less toxicity and having a greater or less affinity for antitoxin. Arrhenius and Madsen hold in opposition to this view that the union between toxin and antitoxin resembles more the union between a weak base and a weak acid. The following is a very brief outline of their argument.

When increasing quantities of a strong acid are added to an aqueous solution of a base the basic quality of the solution gradually diminishes, and finally disappears when the equivalent quantity has been added. Thus if one equivalent of acid be added to four equivalents of base three equivalents of base remain free; if three equivalents of acid be added to four equivalents of base one equivalent of base remains unneutralised. In other words, the amount of base neutralised is proportional to the amount of acid added and this relation can be plotted graphically as the straight line *AN* in Fig. 8 where the ordinates represent the base and the abscissae the acid equivalents.

---

[1] I am much indebted to Mr J. A. Craw for revising the proofs of the sections on the Physico-chemical and Adsorption theories.

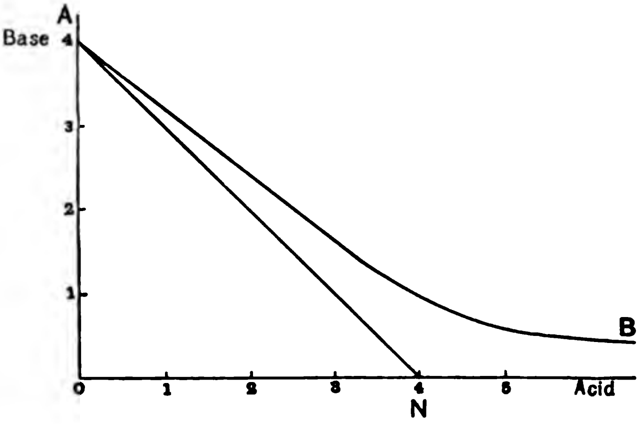


Fig. 8.

This state of affairs strictly holds only for strong acids and strong bases; in the case of weaker acids and weaker bases there is a tendency to the formation of a curve near the neutralisation point *N* where the neutralisation line *AN* passes on to the axis of the abscissae. In reality, even in the case of strong acids and strong bases a slight bending occurs at *N*, but is so slight that it cannot be detected by ordinary chemical means. There is only a quantitative difference between strong acids and strong bases on the one hand and weak bases and weak acids on the other hand.

If the base and the acid are both very weak the bend near *N* becomes very distinct, *e.g.*, the neutralisation of ammonia by boric acid may be represented by the curve *AB*.

As will be seen from the curve (Fig. 9), after the addition of the first equivalent of the boric acid 50 % of the ammonia is neutralised; after the addition of the second equivalent 66·7 %; after the addition of the third equivalent 75 %; and after the fourth equivalent 80 %. Therefore, whereas the first equivalent added neutralised 50 %, the second neutralises only 16·7 %, the third 8·3 %, and the fourth 5 %.

Arrhenius and Madsen carried out a series of haemolytic experiments using ammonia in place of a toxin, such as tetanolysin, and boric acid in the place of the antitoxin, and they found that the results were in every way comparable. By the method of partial saturation of the ammonia with boric acid they found that the first additions of the acid produced a much greater relative diminution in the haemolytic action than did the later additions, just as occurs in the case of tetanus toxin and antitoxin. The curve obtained by plotting out the quantities of uncombined ammonia as tested by their haemolytic action is seen in Fig. 10, and gives an almost exact graphic representation of what occurs in the case of the neutralisation of tetanolysin by anti-tetanolysin. The curve is of the same nature as that representing the equilibrium between a body in partial dissociation and the products of dissociation, in which case the condition of equilibrium has been found to be capable of expression by the equation:

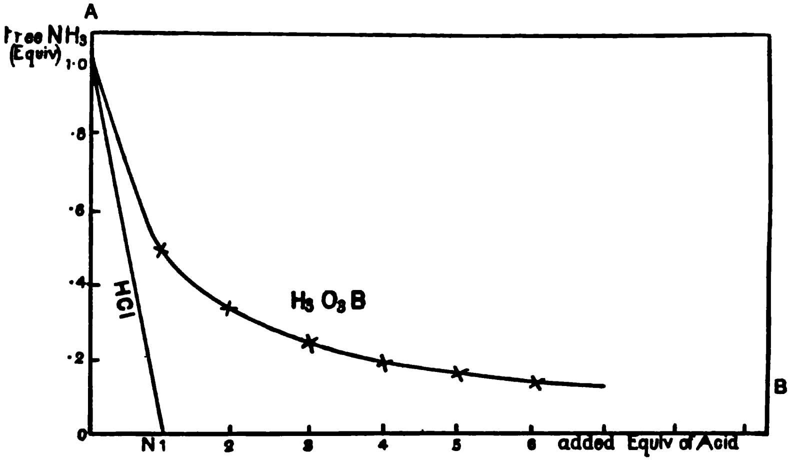


Fig. 9. Curve of Neutralisation of Ammonia by Acids (after Arrhenius and Madsen).

$$\text{(concentration of free acid) (concentration of free base)} \\ = K\,\text{(concentration of combined base)}^2,$$

where K is the constant of dissociation. In the cases of strong acids and strong bases $K = 0$ (or more correctly is of exceedingly small magnitude).

Arrhenius and Madsen say that if Ehrlich's reasoning were applied to this experiment the first amount of ammonia neutralised would be regarded as three times as toxic as the second, the second twice as toxic as the third, and so on. Therefore ammonia is not a simple body but a complex one consisting of several components of different toxicity and different chemical affinities, a *reductio ad absurdum*. The toxin and the ammonia must be equally regarded as simple chemical substances, and the union between toxin and antitoxin, just as that between ammonia and boric acid, must conform to the law of chemical mass-action of Guldberg and Waage, viz., that the amount of the action is proportional to the active mass of each of the reacting substances.

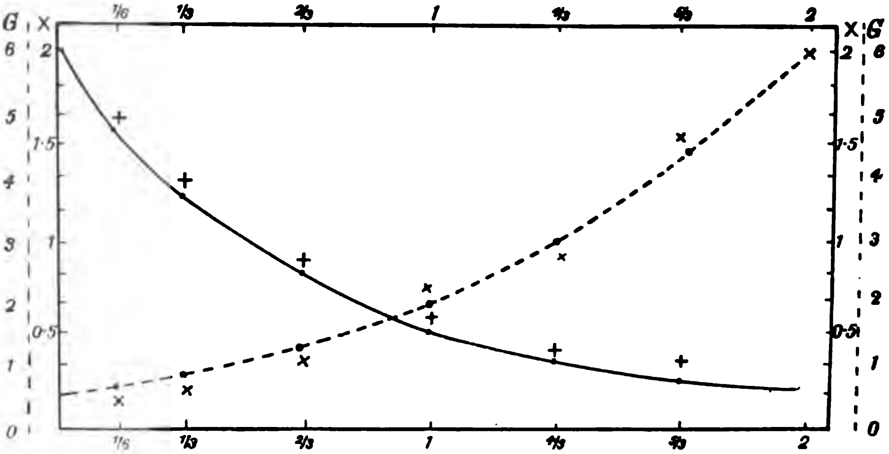


Fig. 10. Curves showing the Neutralisation of the Haemolytic Action of Ammonia by Boric Acid (after Arrhenius and Madsen).

Calculated values plotted as curves:—

+ observed values.

× ,, ,,

—— toxicity of mixture due to unneutralised ammonia (Haemolysin, G).

- - - - quantity of mixture required to produce a certain arbitrary haemolytic effect (X).

A series of mixtures are prepared containing an arbitrary quantity of ammonia (toxin) and increasing quantities of boric acid (antitoxin).

The toxicity of the mixture (G) is thus gradually diminished. X is the quantity which it is necessary to remove from such a mixture to produce, when introduced into a fixed volume of standard suspension of red blood corpuscles, a certain arbitrary haemolytic effect. From this it is clear that X will be inversely proportional to G, as is indicated by the two curves.

The view that toxins are of complex character resulted from the difficulty that attached to the investigation of the first toxin studied, namely, diphtheria toxin. A specially misleading feature in this case was the fact that apparently two forms of intoxication occurred, an acute form resulting in rapid death, and a chronic form resulting in late paralysis. A perfectly natural deduction was that there were present in the diphtheria toxin two separate substances. The second form of intoxication resulted especially when toxin together with a comparatively large amount of antitoxin was present and when such a mixture, even in large doses, failed to produce the acute toxic action.

Diphtheria toxin examined in this new light gives results which are quite in accordance with those obtained in the cases of ammonia and boric acid and tetanolysin and anti-tetanolysin. We can examine a toxin by adding varying quantities of antitoxin to an arbitrary quantity of the toxin and ascertain the toxicities of the resulting mixtures. In such experiments volume may be neglected as the volume of the body of the guinea-pig is practically constant. Just as in the case of ammonia and boric acid, the results may be represented by a curve, which has a close resemblance to the analogous curve of tetanolysin and its antitoxin, in which the ordinates indicate the toxicity of the resulting mixture, or the number of lethal doses in each, and the abscissae the amounts of antitoxin added to the constant quantity of toxin. By proceeding in this way Arrhenius and Madsen found that their results could be expressed by the formula

$$\frac{\text{toxin free}}{\text{vol.}} \times \frac{\text{antitoxin free}}{\text{vol.}} = K\left(\frac{\text{toxin-antitoxin}}{\text{vol.}}\right)^2$$

which had been found to apply to the case of equilibrium between a body in partial dissociation and its products of dissociation.

The equation for this case takes the form

$$\frac{1}{T_0}\left\{np\frac{1}{T}-\left(\frac{1}{T}-\frac{1}{T_0}\right)\right\}=K\left(\frac{1}{T}-\frac{1}{T_0}\right)^2,$$

where K is the dissociation constant which is different for each toxin; n is the quantity of antitoxin added in fractions of an antitoxic unit; T_0 is the number of lethal doses in the quantity of toxin employed where no antitoxin has been added, therefore $\frac{1}{T_0}$ is the minimal lethal dose of that toxin; T is the number of minimal lethal doses in a mixture after the addition of antitoxin, therefore $\frac{1}{T}$ is the minimal lethal dose of that mixture; p indicates the number of toxin equivalents in an antitoxic unit if we take the quantity of toxin employed as an arbitrary unit. K and p are determined from two or more equations of the above form where they are regarded as the two unknowns, the other values in the equations having been ascertained by experiment.

The manner of arriving at the above equation is as follows:

If we inject, for example, a quantity of the mixture sufficient to kill a guinea-pig on the fourth day, in which case the free toxin is equal to 1 M.L.D. or $\frac{1}{T_0}$, then $np\frac{1}{T}$ is the total quantity of antitoxin in the mixture, for $\frac{1}{T}$ is the quantity which contains 1 M.L.D. of the toxic mixture; n of antitoxin was added, therefore $\frac{n}{T}$ is present before combination. Assume that one antitoxic unit is equivalent to p toxic units expressed in M.L.D., then the total amount of antitoxin, expressed as equivalent units, in the lethal mixture is $\frac{np}{T}$. The difference between the quantity added $\frac{1}{T}$, and the quantity free $\frac{1}{T_0}$, that is $\frac{1}{T}-\frac{1}{T_0}$, expresses the quantity of toxin fixed in combination. The basis of the whole theory is that toxin and antitoxin combine in equivalent proportions, therefore the combined antitoxin can also be expressed by $\frac{1}{T}-\frac{1}{T_0}$. The total antitoxin minus the fixed antitoxin gives the free antitoxin, therefore from the above determinations $np\frac{1}{T}-\left(\frac{1}{T}-\frac{1}{T_0}\right)$ expresses the free antitoxin.

Then the equation

$$\left(\frac{\text{toxin free}}{\text{vol.}}\right)\times\left(\frac{\text{antitoxin free}}{\text{vol.}}\right)=K\left(\frac{\text{toxin-antitoxin}}{\text{vol.}}\right)^2$$

can be expressed thus

$$\frac{1}{T_0}\times\left\{np\frac{1}{T}-\left(\frac{1}{T}-\frac{1}{T_0}\right)\right\}=K\left(\frac{1}{T}-\frac{1}{T_0}\right)^2.$$

A few examples of the experiments required to arrive at the necessary data may help to elucidate the matter.

A toxin has 420 M.L.D. in the quantity of toxin employed expressed as unity.

In this case no fraction of the antitoxic unit has been added, *i.e.* $n=0$ and $T_0=420$, so that $\frac{1}{T_0}=\frac{1}{420}=\cdot 0023$. We may tabulate the result thus:

n	T_0	$\frac{1}{T_0}$
0	420	·0023

Now if ·1 c.c. of an antitoxic unit is added to the toxin and the toxicity of the mixture tested on guinea-pigs and it is found that there are only 306 M.L.D. in the mixture and 1 M.L.D. is $\frac{1}{306}$ or ·0032 the result may be tabulated thus:

n	T	$\frac{1}{T}$
0·1	306	·0032

In another experiment where ·2 c.c. of the antitoxic unit is added only 209 M.L.D. are present in the mixture, therefore

$$T=209 \text{ and } \frac{1}{T}=\frac{1}{209}=\cdot 0047,$$

which may be tabulated thus:

n	T	$\frac{1}{T}$
0·2	209	·0047

By substituting these values which have been ascertained experimentally we have the data for two equations

$$(1)\quad \cdot0023\,\{\cdot1 \times p \times \cdot0032 - (\cdot0032 - \cdot0023)\} = K\,(\cdot0032 - \cdot0023)^2,$$

$$(2)\quad \cdot0023\,\{\cdot2 \times p \times \cdot0047 - (\cdot0047 - \cdot0023)\} = K\,(\cdot0047 - \cdot0023)^2,$$

from which the values of p and K can be determined mathematically.

Where only two such equations are employed to ascertain the values of p and K these will be only approximate, but the more equations, based on experimental data, employed the more accurate will be the values. p and K are therefore determined by Arrhenius and Madsen from as many data as possible and at the same time the corrections of the M.L.D. as ascertained from loss of weight and lethal period are employed (see p. 470).

The most accurate values possible for p and K having been determined the equations are then employed to arrive at the theoretical values of T.

A complete series of experiments by Madsen of the nature indicated are shown in the Table.

Neutralisation of Toxin No. 471 (Arrhenius and Madsen). Ehrlich's Standard Test Serum was employed.

$p = 2\cdot5$ $\qquad K = 0\cdot0122$

n	$n \times p$	T	Observed	Calculated
0	0	420	100	100
0·05	0·125	313	74·4	87·5
0·1	0·25	306	72·8	75·1
0·15	0·375	242	57·6	62·7
0·2	0·5	209	49·8	50·6
0·25	0·625	135	32·2	38·6
0·3	0·75	118	28·0	27·3
0·35	0·875	72·5	17·2	17·5
0·4	1·00	46·5	11·1	9·9
0·45	1·125	23·5	5·6	6·0
0·5	1·25	5	1·2	4·1

Note. K is the dissociation constant. T = the toxic units or minimal lethal doses in the mixtures. n is the fraction of an antitoxic unit which is added to the toxin. The constant quantity of toxin employed in these experiments being taken as unity, the value "p" indicates the number of equivalents of antitoxin contained in one antitoxic unit—

$$\frac{p}{1} = \frac{\text{immunising unit}}{\text{toxic unit}}.$$

If n parts of the antitoxic unit be added to this unit of toxin then the product "np" indicates the ratio between the equivalents of antitoxin and toxin in the individual cases examined

$$\frac{np}{1} = \frac{\text{equivalents of antitoxin}}{\text{toxin unit}} .$$

In the 4th and 5th columns under the bracket, the calculated and observed values of T are reduced to percentages.

The numbers obtained by experiment and calculation were found to be in agreement within the experimental error.

In the accompanying chart the observed and calculated values reduced to percentages from the above table are plotted. The ordinates show the number of lethal doses, the abscissae the amounts of antitoxin added to the constant quantity of toxin employed.

Chart showing the percentage values of T, observed values indicated thus ⊙, calculated values thus ● on the curve.

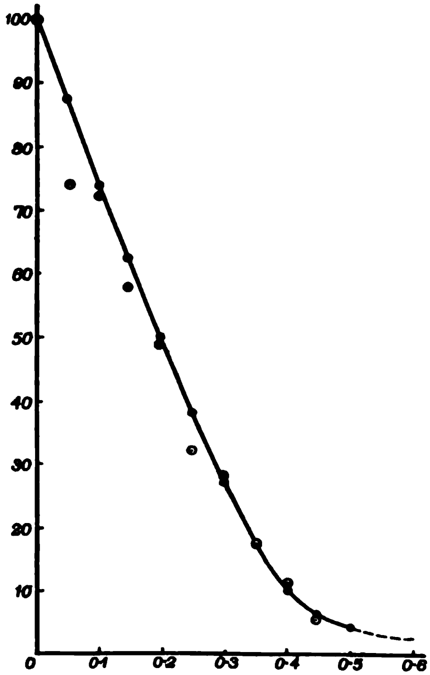


Fig. 11. The ordinates indicate the number of lethal doses in the mixtures (T), the abscissae the fractions of a c.c. of antitoxin added.

Toxicity of mixtures of Diphtheria Antitoxin and Diphtheria Toxin at Different Dates.

$p=2·5$ $K=0·0122$.

		T. observed (Feb. 1902)		T. observed (Sept. 1903)		
n	$n \times p$	100	T.	100	T.	T. calculated 100
0·10	0·25	72·8	306	75·1	161	75·1
0·15	0·375	57·6	242	62·6	134	62·7
0·20	0·5	49·8	209	47·6	102	50·6
0·25	0·625	32·2	135	45·8	98	38·6
0·30	0·75	28·0	118	25·9	55·5	27·3
0·35	0·875	17·2	72·5	17·3	37	17·5
0·40	1·00	11·1	46·5	9·6	20·6	9·9
0·45	1·125	5·6	23·5	5·3	11·4	6·0
0·50	1·25	1·2	5·0	3·1	6·5	4·1
0·60	—	—	—	1·6	3·5	2·6

The toxin employed lost half its strength in 17 months though it maintained its power of fixing antitoxin, and the value of K remained quasi-constant. To explain this phase Madsen and Arrhenius have admitted, following Ehrlich, that the poison has been transformed into an inoffensive modification, "syntoxoid," possessing the same affinity for antitoxin, and the constant of equilibrium of the initial poison. If we take this view of the matter we must admit that the two molecules which result from the reactions of the toxin and of the toxoid with the antitoxin are different.

The reaction recalls that of etherification:

$$\text{acid} + \text{ethylic alcohol} \leftrightarrows \text{water} + \text{ether}.$$

If this is the case the reaction resembles an etherification in which two acids having the same affinity for an alcohol react on it, and give rise to two different ethers and water. The analogue of the water, the common substance resulting from the reaction, they call titoxin; of the analogues of the ethers, the one resulting from toxoid they call toxoidane, the other resulting from toxin they call toxinane.

Toxin and antitoxin = toxinane + titoxin.
Toxoid and antitoxin = toxoidane + titoxin.

From the formula it appears that one molecule of toxin combines with one molecule of antitoxin to form two molecules of the new combination toxin-antitoxin. If to a given quantity of toxin there is added a relatively small amount of antitoxin this will be almost entirely fixed and the surplus of the toxin will remain free. There will be almost no antitoxin free because in these proportions the combination toxin-antitoxin is only very feebly dissociated. As we increase the amount of antitoxin a greater amount of toxin will be fixed, but at the same time the toxin-antitoxin will be dissociated more and more, so that there will always be present toxin and antitoxin side by side. If incomplete association of the combination toxin-antitoxin did not take place then the union between toxin and antitoxin would be in the form of a straight line as in the case of the strong acid and the strong base.

As we add more and more antitoxin the quantity of the free toxin diminishes but the decrease occurs progressively more slowly, and theoretically however much antitoxin may be added there remains always some toxin free. This phenomenon is indicated by the form of the curve. The difficulty of determining the L_0 and the point of complete neutralisation can be explained from this point of view since such a neutral point does not exist. In this way also the toxone-zone of the spectrum may be explained. The slight oedema and late paralysis, according to Arrhenius and Madsen, are due not to a separate body toxone but to toxin either unneutralised or dissociated.

The dissociation of the toxin-antitoxin compound gives a comparatively easy explanation of the results obtained by Dreyer and Madsen in the rabbit and guinea-pig. A mixture of toxin and antitoxin, which had no effect on the guinea-pig, when injected intravenously into the rabbit produced paralysis; and a mixture, which in the guinea-pig gave rise to paralysis only, was rapidly fatal to the rabbit. It is difficult to understand this result if we regard the paralysing substance, the toxone, as a separate body, whereas it is easy to understand and interpret if we attribute both acute death and paralysis to the toxin and regard the difference as only a quantitative one.

Madsen and Walbum (1904) have obtained with neutral mixtures of diphtheria toxin and antitoxin results which they regard as an absolute proof of the occurrence of dissociation. They pour on to the surface of a column of gelatin a mixture of diphtheria toxin and antitoxin, which is absolutely harmless to a guinea-pig. After remaining for 66 days at a temperature of 2° to 3° C., half a c.cm. of the column of gelatin is cut off, melted and mixed with a lethal dose of toxin. This mixture injected into a guinea-pig does not kill the animal. Some antitoxin, therefore, has diffused into the upper layers of the gelatin. The 10 c.cm. below this upper layer is melted and one half of it when injected into a guinea-pig kills the animal; the

Chart showing the theoretical curve of neutralisation of diphtheria toxin by increasing quantities of antitoxin, contrasted with certain experimental data (after Madsen, 1903, *p.* 640).

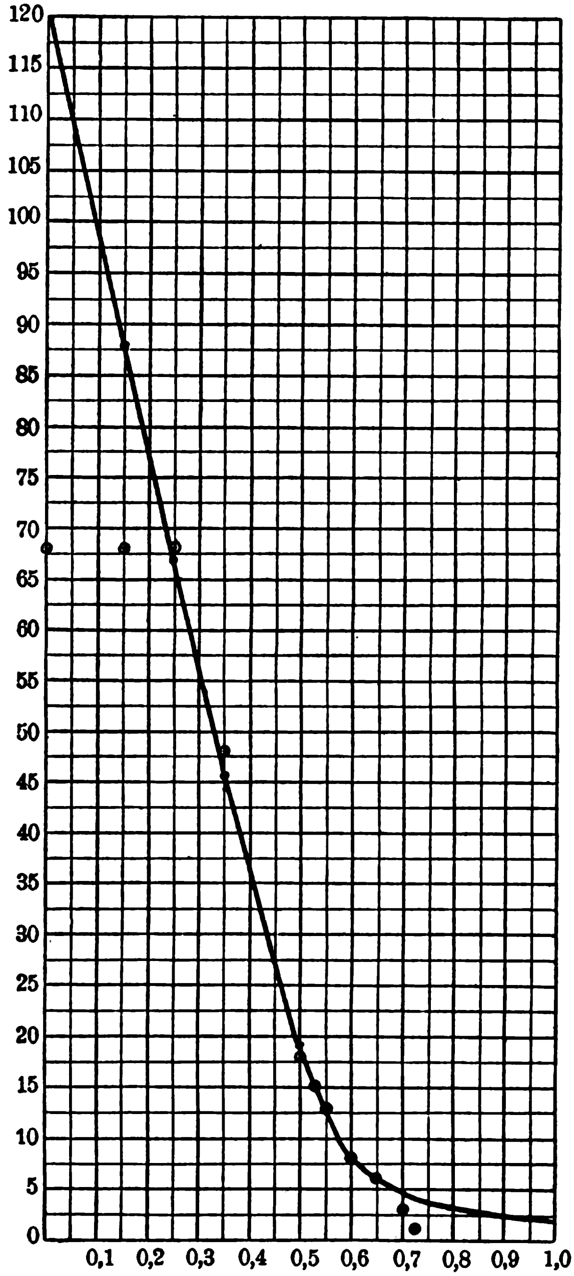


Fig. 12. The ordinates indicate toxicity or number of lethal doses, the abscissae the amounts of antitoxin added to the constant quantity of toxin.

● = calculated values. ⊙ = observed values.

other half, mixed with antitoxin, produces in another guinea-pig only a slight transient oedema, probably due to the gelatin. The interpretation of this experiment appears to be that a neutral mixture of diphtheria toxin and antitoxin has become dissociated, part of the dissociated antitoxin has diffused into the upper layer of gelatin, and the dissociated toxin being more diffusible has extended deeper into the gelatin.

By a similar method the constants of diffusion for diphtheria toxin and antitoxin, and for tetanolysin and anti-tetanolysin, were estimated with the result that the constants for the antitoxin were found to be much smaller than those for the toxins, indicating that the molecular weights of the antitoxins are much greater than those of the toxins.

Certain of the diphtheria curves worked out by Madsen do not coincide throughout their whole length with the theoretical curves. The upper part of the curve is quite flat and runs parallel to the axis of the abscissae (see Figs. 12 and 13).

Chart showing the toxicities of the mixtures obtained by gradually adding increasing quantities of antitoxin to a fixed quantity of diphtheria toxin (*after Madsen*, 1903, *p.* 639).

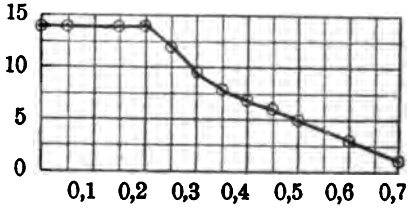


Fig. 13.

This indicates that during the process of partial saturation the first additions of antitoxin to a fixed quantity of toxin have no effect in diminishing the toxicity. Such a result could be explained by retaining Ehrlich's "Protoxoids," but in their more recent communications Arrhenius and Madsen state that in the case of certain toxins this appearance of the curve is absent and that it was probably due to an experimental error.

Arrhenius and Madsen have summed up their results and views in regard to diphtheria toxin and antitoxin as follows:

1. The neutralisation of diphtheria toxin by antitoxin follows the law of Guldberg and Waage.

2. Its constant of dissociation is comparatively insignificant, it varies in the observed cases between 0·004 and 0·03.

3. A toxin in losing its toxicity by age probably follows the law of the rate of monomolecular reactions.

4. That rate of reaction can be very different in different cases so that the stability of one toxin can be double that of another.

5. By its loss of toxicity the toxin does not at first lose to an appreciable degree its power of fixing antitoxin nor does it change its constant of dissociation.

6. No indications of the presence of protoxoids have been found although they have been purposely looked for.

7. It is no longer necessary to believe in the presence of toxones; nevertheless, concentrated solutions of toxin show perturbations due probably to a marked fall in the rapidity of reaction in the presence of high concentrations of antitoxin.

8. All these characteristics of diphtheria toxin can be explained on the following hypotheses:

A. Diphtheria toxin is a homogeneous toxin.

B. Diphtheria toxin changes slowly after the laws of monomolecular reactions into the atoxic body, toxoid.

C. The quantity of toxoid formed by a given quantity of toxin is here considered as equivalent to the toxin. Toxins and toxoids react in equivalent quantities with equal quantities of antitoxin, so that from one molecule of toxin or of toxoid and one molecule of antitoxin there are formed two molecules, the one of a body which is alike in the two cases (called "titoxine"), the other of a body which is not identical in the two cases, called "toxinane" when originating from toxin, and "toxoidane" when from toxoid. Like antitoxin these products are atoxic.

D. The constant of equilibrium is equal for the two reactions, namely, those of the toxin and of the toxoid with the antitoxin.

CRITICISMS OF THE VIEWS OF ARRHENIUS AND MADSEN.

In replying to these views Ehrlich (1903, 1904) adheres to his previous position. He reviews a number of the facts and analyses many of the data of which support his views of the complex composition of diphtheria toxin, and of the nature of the union which takes place between toxin and antitoxin.

In regard to the complexity of diphtheria toxin he says that this falls into line with a common biological experience, that organisms never produce a single characteristic substance but tend rather to give rise to a series of bodies which resemble one another. He instances opium which contains nearly 20 alkaloids, morphine, kodeine, etc., which have a certain similarity of action, but which are sharply differentiated from one another. Cinchona bark furnishes another good example.

He reviews his grounds for considering toxone a separate poison, secreted as such by the bacillus and not derived secondarily from the toxin. The striking difference in the time at which the action occurs and the widely different effects produced on the animal body are strongly in favour of there being two distinct substances at work. There is first the toxin effect which produces death within a few days or in sub-lethal doses gives rise to rapid local symptoms of inflammation in the subcutaneous tissue, oedema, alopecia, accompanied by loss of weight, etc. If on the other hand a man or animal has survived an attack of diphtheria, even if the local effect has been of the slightest, there may supervene after an interval of weeks characteristic paralyses. These late effects are of such a different character that Ehrlich holds they strongly support the view that they are produced by another poison, namely, the toxone.

Further, he refers to his observation that old diphtheria toxin, although its toxicity may have greatly diminished, still requires as much antitoxin as formerly to neutralise it. This can only be explained by the conversion of a part of the toxin into a less toxic substance, "toxoid," which retains its original avidity for antitoxin. The validity of this last argument has, indeed, been admitted by Arrhenius and Madsen. Ehrlich's analyses of the results obtained from the examination of the same toxins at different periods, and of toxins of widely different composition, are too elaborate for reproduction here and we must refer the reader to his original papers and those of Morgenroth (1904).

In addition to the biological arguments against the view that toxins are simple bodies, certain criticisms have been raised touching the physico-chemical views advanced by Arrhenius and Madsen.

If Arrhenius and Madsen's view, that the union between toxin and antitoxin resembles an etherification, is correct, then the toxin-antitoxin reaction must be reversible, for this is the essential feature of such a reaction; the acid acts on the alcohol and gives rise to ether and water; the water again reacts on the ether, and alcohol and acid result.

In such reversible reactions when the same quantities of the reacting substances are employed, no matter how they are brought together, the same equilibrium is established, that is to say, at the end of the reaction the same amounts of the free substances and of the products of reaction are always present in the mixture. Is the equilibrium of such a character in the toxin-antitoxin reaction as to indicate that the reaction is reversible?

Von Dungern (1904), reverting to an observation made by Danysz (1902), investigated this point. Danysz had found that it was not a matter of indifference whether the toxin was added altogether or in fractions to the antitoxic unit. If the L_0 dose of toxin is added to the unit and left in contact for six hours and then more toxin is added it requires a smaller addition of toxin to reach the L_+ dose than if all the toxin were added at once to the antitoxic unit. The difference between the L_+ and the L_0 is smaller, therefore, in the case of fractional addition. Von Dungern confirmed this fact for diphtheria toxin, and indicated its importance as an argument against the reaction being a reversible one and in favour of Ehrlich's assumption of the existence in diphtheria toxin of groups, having different avidities for the antitoxin.

The results obtained by this line of experiment also indicated that a closer binding of the combination occurred after union had taken place. The experiments of Danysz and von Dungern were repeated and confirmed by Sachs in the case of tetanus toxin.

Nernst (1904) has submitted some of the calculations of Arrhenius and Madsen to a searching examination. He finds that the selection of the figures in estimating the end points of reactions has been altogether arbitrary. The equilibria which they assume to exist in the case of toxin-antitoxin are not true equilibria but belong to the class of "false" equilibria, well known to the physical chemist. The variation of the dissociation constant from one diphtheria toxin to another seems to him to demonstrate the complexity of the toxic fluid. He concludes that the law of mass action (Guldberg and Waage) has been improperly applied. This law has hitherto been limited to gases and to true solutions, and this attempt by Arrhenius and Madsen to apply it to substances which are of the nature of colloidal solutions is not justified. In the case of toxins and antitoxins we are dealing not with true solutions, but with a form of suspension, the physico-chemical knowledge of which is only in its infancy. The physical chemist must have a more accurate knowledge of the laws governing the colloidal state before he can hope to deal successfully with biological problems such as the nature and relation of toxin and antitoxin.

Sachs (1904) discusses the question of reversibility. He refers to Martin and Cherry's work in which it was shown that although toxin alone passes through a gelatin filter no toxin was found to pass from a toxin-antitoxin mixture, showing that no free dissociated toxin was present in the mixture. He holds that Madsen and Walbum's experiment of the diffusion of toxin into gelatin is completely upset by Morgenroth's (1904) observation that 24 hours are required for complete union of toxin with antitoxin. Madsen and Walbum say in regard to their experiment that it is a necessary condition for dissociation that the mixture of toxin and antitoxin should be fresh and should not have stood long. Madsen and Walbum's experiment is only an additional proof, therefore, of the irreversible nature of the combination.

THE ADSORPTION VIEWS OF THE TOXIN-ANTITOXIN REACTION.

Bordet (1901—3) thinks that the fixation of toxin by antitoxin is a process analogous to the fixation of a dye on a tissue. He states that his view has been misunderstood, and that by such fixation he does not mean mere mechanical action to the exclusion from the reaction of all chemical characteristics. He holds it would be hazardous to assume, as some of his critics do, that the phenomena of coloration never come within the range of chemistry, but are purely mechanical depending on physical properties, such as the texture and porosity of the stained material. He instances the absorption of iodine from a watery solution by starch.

He holds that the reaction differs from ordinary chemical reactions in that the toxin and antitoxin by their union do not always form one and the same substance which invariably possesses the same characters. The toxin and antitoxin unite in variable proportions, and the molecules of antitoxin distribute themselves on all the toxic molecules present. A complex is thus formed which is less toxic. The phenomena of intoxication in the case of such toxins modified by antitoxin will not be the same as those resulting from the action of intact toxins.

Between the extremes of pure toxin and toxin entirely saturated all the transition stages of progressive attenuation can be conceived.

Every time the same proportions are used the same degree of toxicity of the compound will be attained.

Bordet thinks that a number of anomalous phenomena can thus be explained. It is easy to understand from this view how a mixture neutral for one species of animal may be toxic for another. The toxone phenomenon can also be explained on this hypothesis of compounds having different toxic actions.

Danysz (1902) holds a view identical with Bordet's, and in support of it has added interesting experimental evidence, to which reference has already been made.

Nernst (1904) pointed out that, since the substances involved in the reaction are both colloidal in character, the union is probably of the nature of an adsorption of the smaller toxin molecules by the larger antitoxin molecules.

Craw, J. A. (1905), in a series of experiments on megatheriolysin and antilysin, came to the conclusion that the removal of lysin from a solution by antilysin is not capable of interpretation as a purely chemical change, and that the law of chemical mass action does not apply when the lysin is present in excess. Lysin and antilysin in the free state are present both in sub-neutralised and over-neutralised mixtures. He holds that the phenomena are more analogous to certain adsorption phenomena.

THE BEHAVIOUR OF ANTITOXIN IN THE ANIMAL BODY WHEN USED FOR THE PRODUCTION OF PASSIVE IMMUNITY.

When diphtheria antitoxin is injected subcutaneously with the view of rendering an animal immune, a certain time must elapse before the maximum effect is attained. Owing to the large size of its constituent particles the antitoxin diffuses somewhat slowly, first into the lymphatics and thence into the blood stream. So slow is the passage from the site of inoculation into the general circulation that, according to Behring (1901), eight hours can be saved by injecting the antitoxin directly into the blood stream. Henderson Smith (1907) found that after a subcutaneous injection of diphtheria antitoxin the maximum antitoxic value of the blood serum was not reached till about the third day. Apart from its specific action, by means of which the recipient of the serum is rendered immune to the disease, certain secondary collateral effects are sometimes produced which will be dealt with in Chapter XIX.

When the antitoxin is injected into persons already affected with diphtheria it combines with any toxin which may be present in the blood and prevents the poison from attacking the susceptible cells. So long as there is free antitoxin in the blood the toxin elaborated at the site of infection and absorbed into the blood does not reach the cells for which it has a specific affinity, but is arrested and neutralised by the antitoxin, and the latter may even have some influence in wresting the toxin from the cells during the early stages of combination. The experiments of Fraser (1896), C. J. Martin (1898), and Heymans (1898) and Dönitz (1899) are interesting in this connection. These observers find that the combination between the toxin and the susceptible cells takes place very rapidly, and that therefore a very short interval only must elapse between the injection of the toxin and the subsequent injection of the antitoxin, if the latter is to be employed to prevent the union. Dönitz, for instance, found that if a single lethal dose of toxin was injected intravenously into a rabbit before the antitoxin, it could be neutralised by the same amount of antitoxin as was required *in vitro*, provided an interval of not more than nine minutes elapsed. If the interval was longer, then a larger dose of antitoxin was necessary to save the life of the animal. If a period of one and a half to two hours, or longer, had passed after the toxin injection, then no amount of antitoxin was capable of averting the death of the animal. The natural interpretation of these results is that the toxin at first circulates free in the blood, during which time the toxin-antitoxin combination takes place just as *in vitro*; later the toxin becomes anchored on to the susceptible cells, but up to a certain period the combination is a loose one, capable of being broken down by the presence of a large quantity of antitoxin; finally the toxin attaches itself so firmly to the cells that no matter how much antitoxin is used the union cannot be dissolved.

The Rate of Disappearance of Antitoxin from the Blood.

The larger the amount of antitoxin injected into an animal the longer, *caeteris paribus*, will antitoxin be capable of demonstration in the blood. The rate of disappearance according to Bomstein (1897) and others is proportional to the concentration of the antitoxin in the blood.

The duration of passive immunity in diphtheria is one of great practical importance and has been studied by a number of observers. Behring (1897) found that there was still a considerable quantity of antitoxin present in the blood of goats 23 days after a large injection, and that antitoxin was demonstrable in the blood of children up to four weeks after a dose of 250 units. This coincides with a wide clinical experience that protection lasts for about four weeks. Passini (1896), after an injection in man, failed to demonstrate antitoxin after 12 days. Bomstein (1898) injected 8000 to 10,000 units into dogs, and failed to find any antitoxin after 18 days. Bulloch (1898) injected 25,000 units into a donkey and traced the antitoxin curve. This reached its maximum in 24 hours. On the fourth day a marked fall was noted, which continued until the 100th day when antitoxin in a measurable quantity was still present. On the 126th day it was below one-tenth of a unit per c.c. Kraus and Joachim (1903) have shown that when diphtheria antitoxin, obtained from the horse, is injected into the rabbit its disappearance is very rapid, a great diminution being discernible even within one hour[1].

Absorption of Antitoxin from the Alimentary Canal.

Ehrlich (1892) clearly demonstrated that suckling animals could absorb antitoxin from the mothers' milk, and this fact has been confirmed by a number of workers. Escherich (1897) investigated the question as to whether antitoxin could be absorbed when administered *per os* or *per rectum.* The experiment was carried out on children of various ages, but the only positive results were obtained when the antitoxin was administered *per os* in sucklings. Rectal injection gave entirely negative results. Since Escherich's work many papers have appeared claiming good clinical results from the administration of antitoxin by the mouth, but experimental results show that there is little or no absorption of the antitoxin obtained from another species. Thus Salge (1904) found that when the antitoxin was an integral part of the milk it could be demonstrated by Marx's (1901) method in the blood of sucklings. When, however, antitoxic serum from the horse was added to the milk, antitoxin could not be found in the blood. Salge concluded that the homologous proteids of the mother's milk could be absorbed by the child, whereas the heterologous proteids of the serum of the horse could not pass through the walls of the alimentary canal. Uffenheimer (1904), who reviewed Salge's paper, said that his own experiments on animals (the antitoxin being measured by Marx's method) gave results in opposition to those of Salge. Dzierzgowski (1899) found that when the antitoxin was introduced into the empty stomach of the rabbit a slight immunity was conferred. Hewlett (1902) could find no trace of absorption of antitoxin from the stomach or rectum in the case of guinea-pigs and rabbits. The factors which hinder diphtheria toxin, introduced into the alimentary canal, from exercising its toxic action are no doubt at work in preventing the absorption of the antitoxin. The hydrochloric acid of the gastric juice probably plays an important part. Oppenheimer states that neutralised pepsin, pancreatin and bile are not very harmful. Pick found that two-thirds of the antitoxin was destroyed during a trypsin digestion experiment lasting nine days. Intestinal bacteria probably exert a destructive influence on the antitoxin. Though colloidal substances with large molecules diffuse with difficulty through an animal membrane, this does not prevent the passage of antitoxin in the suckling. It is possible, however, that the homologous or heterologous nature of the antitoxic serum may be an important factor in influencing absorption.

---

[1] This subject is closely associated with the question of the production of anti-antitoxin dealt with in another section, p. 568.

THE QUESTION OF THE PRODUCTION OF ANTI-ANTITOXIN.

Does an antitoxic serum obtained from one species, when injected into another species, give rise to the production of an anti-antitoxin? The rapid disappearance of antitoxin from the blood of passively immunised animals would be readily reconcilable with such an hypothesis.

Pfeiffer and Friedberger (1902 and 1903) were able to demonstrate that an anti-antibody could be obtained to a bacteriolytic serum. The injection into rabbits of a cholera-immune serum from the goat gave rise to the formation of an anti-bacteriolytic serum with "anti-cholera amboceptors."

From this observation it might have been anticipated that the demonstration of an anti-antitoxin would be an easy matter. This, however, is not the case, and a number of observers, among whom may be mentioned Kraus and Eisenberg (1902), and Kraus and Joachim (1903), have reported experiments which show that in the case of diphtheria the injection of a heterologous antitoxic serum into an animal does not give rise to an anti-antitoxin.

Certain observations relating to the production of specific precipitins bear on this subject. Thus Atkinson (1903) showed that the sera of rabbits, some of which had been injected with normal horse serum, others with antitoxic serum, in both cases produced precipitins which were capable of throwing down the substance to which the antitoxin adheres. Sacharof (1905) also found that diphtheria antitoxin in horse serum is attached to the elements which give rise to precipitin formation, and that the precipitate obtained from serum by the use of this precipitin includes the antitoxin adhering to the proteids. That a similar process occurs in the animal body was shown by the following experiment. Into two rabbits of equal weight, of which one was normal and the other had previously received an injection of normal horse serum, the same quantity of diphtheria antitoxin was injected. After a short time the animals were bled and their serum was tested for the presence of antitoxin against a certain dose of toxin. It was found that a larger quantity of serum from the animal which had received the injection of normal horse serum was required in order to neutralise the dose of diphtheria toxin.

It must be noted that these results do not prove the existence of a substance which acts *per se* as an anti-antitoxin; the apparent action in that direction may be merely a secondary effect of the precipitin action.

INHERITED IMMUNITY.

Transmission of acquired immunity to offspring.

Ehrlich (1892) and Ehrlich and Hübner (1894) first successfully employed experimental methods in investigating the transmission of acquired immunity from the parent to the offspring. A series of experiments to investigate the transmission of immunity in the case of abrin, ricin, and robin was carried out in mice. Later, similar investigations were made in regard to immunity to tetanus toxin, and for these experiments mice and guinea-pigs were employed.

In these experiments Ehrlich used the ingenious method of transferring the young of the normal mouse to an immune foster-mother, and the young of an immune mother to a normal foster-mother. The conclusions arrived at were summed up as follows.

1. The idioplasm of the sperma cannot transfer immunity.
2. The ovum is also incapable of transferring immunity.
3. The immunity which can be observed in the progeny at birth originates from maternal antibodies received through the placental

blood. The immunity is of short duration, lasting up to three to four weeks.

4. The milk is the actual agent by which the immune substances are conveyed to the suckling.

5. The longer the maternal milk is supplied the greater is the immunity attained.

6. The immunity is, therefore, passive and is limited to two or three months.

Wernicke (1895) confirmed Ehrlich's results in the case of diphtheria immunity in the guinea-pig.

On the other hand, a number of workers have supported the view that a true, inherited, histogenic immunity can occur. Vaillard (1896), for example, came to the conclusion that the leucocytes of the descendants of animals, which had been immunised against anthrax, inherited a specially active phagocytic power against that bacillus. Tizzoni and Centanni (1893), working with the virus of rabies, and Gley and Charrin (1893), working with *B. pyocyaneus*, have asserted that the immunity can be propagated from the male to the offspring by means of the sperma.

Remlinger (1899) concluded that in regard to the transmission of immunity against the typhoid bacillus the male does not exert any influence, but that the rôle of the female is important, especially when the immunisation has been carried on during gestation; even then the immunity is transient, lasting only one or two months, and is limited to the one gestation after the vaccination. He thought that suckling did not, at least in the case of the rabbit or guinea-pig, play any part in the transmission of immunity.

On the question of the transmission of the agglutinating power there is a mass of literature into which we cannot enter here. In Remlinger's experimental investigations it was found that the male animal played no part, and that it was only when the injections of bacilli were carried out during gestation that the young acquired the property through the placental circulation. The agglutinating property could not be acquired through suckling.

Kraus (1901) was the first to study the transmission of immunity from parents to offspring by the employment of haemolysins obtained by injecting the red blood corpuscles of an animal into an animal of a different species. He came to the conclusion that as no immune substance could be demonstrated in the milk, and that as the serum of the young of the immune rabbits was haemolytic, the haemolysin must pass to the young through the placental circulation. Bulloch (1902), on the other hand, found that immune substance, but no complement, was present in the milk of immunised rabbits. His results entirely confirmed Ehrlich's observations on this subject.

Dzierzgowski (1901) carried out a series of experiments on the inheritance of acquired diphtheria immunity, using hens for his experiments. The immunity was produced by first injecting antitoxin only, and later combined toxin and antitoxin. During the time when serum alone was being used the eggs laid contained no antitoxin, but in those laid after toxin and antitoxin had been injected antitoxin could be demonstrated in the yolk. The antitoxin was found to be associated with a form of globulin, as in the case of the blood serum. The blood of the chicks hatched from some of these eggs contained antitoxin. Dzierzgowski regarded the immunity as of a purely passive character.

THE QUESTION OF ANTI-MICROBIAL DIPHTHERIA SERUM.

From the time of the appearance of Behring's first papers on diphtheria immunity it was recognised that anti-diphtheritic serum had anti-microbic, as well as antitoxic, properties. Behring's observation was confirmed and extended by a number of observers, among whom may be mentioned Klein and Cobbett.

Van de Velde (1897) undertook an investigation to ascertain whether the two properties of the serum could be obtained separately by different methods of immunisation. He immunised five goats by different methods, and the following Table gives a brief outline of the results obtained. In testing the efficacy of the serum in protecting

Goat	Goat treated with	Guinea-pigs treated with serum of goat and afterwards injected with	Result
1.	Strong toxin	1. Toxin	Survived.
		2. Bacilli	Survived.
2.	Washed culture, killed by thymol	1. Toxin	Died in 2½ days.
		2. Bacilli	Survived.
3.	Culture in bouillon; bacilli (killed by thymol) + toxin	1. Toxin	Lived.
		2. Bacilli	Lived.
4.	Weak toxin	1. Toxin	Died in 5 days.
		2. Bacilli	Lived.
5.	Toxin heated to 100° C. for 1 hour.	1. Toxin	Died in 36 hours.
		2. Bacilli	Died in 2½ days.

guinea-pigs he used threefold lethal doses of toxin, or of bacilli free from toxin. The serum was injected into one side of the body and 12 hours later the toxin or bacilli into the other side.

He was thus able to show that the two properties were essentially different, and could be obtained separately according to the method of immunisation employed. He found that in the antitoxin of commerce both the antitoxic and the anti-infectious properties were present. He was unable to separate these by heating the serum to various temperatures.

Lipstein (1903) in re-investigating the question as to whether it is possible to obtain an anti-microbial diphtheria serum indicates that the work of Van de Velde, Klein and others is open to the criticism that their results were perhaps due to the antitoxic, and not to the anti-microbic properties in the serum. He refers to the universal experience that antitoxin is itself capable of protecting against the living organism, and holds that Bandi (1902), who found that various commercial antitoxins did not protect animals against his bacillus, could not have employed a true diphtheria bacillus for his experiments.

In order to neutralise all traces of diphtheria toxin which, as was shown by Kossel, Brieger and Boer, and others, adhere to or are contained in the bacillary bodies Lipstein added diphtheria antitoxin to emulsions of virulent diphtheria bacilli, which had been grown on nutrient agar, and immunised rabbits and guinea-pigs with these mixtures. As the result of a very thorough series of experiments he concluded that in the case of the animals employed by him there was no amboceptor production, and therefore no bactericidal action either *in vitro* or *in corpore*, and that the survival of certain of his experimental animals was due to the traces of antitoxin, sufficient in themselves to afford protection as shown by control experiments.

Fresh interest has recently been aroused in the question of an anti-microbial serum by the work of L. Martin (1903). By injecting the bacillary bodies intravenously or intraperitoneally into a horse he obtained a serum with marked agglutinating properties (see p. 226). He claims that this serum has the property when applied locally of causing a rapid decrease in the number of living bacilli in the throat, and that coincidently the local pain quickly disappears and the false membrane becomes more rapidly detached than when only antitoxic serum is used.

The best results are obtained by incorporating the dried serum with gum and using in the form of pastilles. The great value of such a serum would lie in the reduction by its means of the duration of the period of isolation and residence in hospital, so often necessitated by the persistence of the bacilli in the throats of patients long after recovery from all signs of disease.

Some observations on this subject were made by the Serum Department of the Lister Institute, two horses being injected intravenously first with killed and then with living bacilli. The sera obtained agglutinated the race of bacillus which was employed in dilutions of 1 : 200 and 1 : 300. Clinical tests were made on a series of cases with pastilles containing the serum. No shortening of the period of persistence of the bacilli in the throat was found.

SECTION VI.

CHAPTER XVIII.

MORTALITY.

By WILLIAM H. PARK AND CHARLES BOLDUAN.

The history of mortality from diphtheria before and after the introduction of the antitoxin treatment. The effect upon statistics of bacteriological examinations of suspected cases. The proportion of diphtheria cases sent to hospitals. The influence of the day of admission on the prognosis of hospital cases. The effect of heat and cold on the prevalence of diphtheria. The influence of altitude and soil on the prevalence of diphtheria. The influence of race, rainfall, and occupation. The mortality and prevalence of diphtheria at different ages. Infectivity and virulence. Characteristics of epidemics. Results of antitoxin treatment. Comparison of case mortalities. The influence of the time of administration on the value of antitoxin treatment. Regarding a possible decline in the virulence of the disease in recent times. Operative cases. Statistics on alternate cases. The absolute mortalities during a long series of years in large cities as an index of the value of antitoxin treatment. The extent to which antitoxin is used.

THE HISTORY OF THE MORTALITY FROM DIPHTHERIA IN VARIOUS OUTBREAKS, AND OF THE MORTALITY BEFORE AND AFTER THE INTRODUCTION OF THE ANTITOXIN TREATMENT.

BEFORE entering on a consideration of the mortality from diphtheria it seems wise to consider certain facts whose proper understanding is necessary to make this study of any value.

The history of the disease now known as diphtheria goes back to a remote time, long before any accurate records were kept. Even during recent years the disease in its different localisations in the body has been known under different names, and this fact must be recognised in order to avoid faulty conclusions.

In 1765 Francis Home, of Edinburgh, published a pamphlet entitled "An Inquiry into the Nature, Cause and Cure of Croup." The word "croup" at that time was the term given to diphtheria when the larynx was attacked, the Scotch word "croup" meaning to croak or to speak with a harsh voice. Home's account of the autopsy findings accurately describes such cases as they are met with at the present time, and is extremely interesting.

In 1771 Samuel Bard, of New York, wrote an elaborate article on the "Cause, Nature and Treatment of Suffocative Angina." His description of the disease, which we at once recognise as diphtheria, was extremely vivid, but his knowledge of the etiology was somewhat vague.

The present name was given to the disease by Bretonneau in 1821. His observations were so extensive and so correct that little further advance in knowledge took place until the causal relations of the diphtheria bacilli and their associated micro-organisms to the disease began to be recognised.

In the official death returns in New York we find the term diphtheria first used in 1857, when two deaths due to this cause were reported.

The following table of deaths, from the New York City records, is of interest because it gives the names by which the disease was formerly known, and shows how the use of the term diphtheria rapidly spread.

TABLE I.

	Inflammation of throat	Inflammation of tonsils	Quinsy	Sprue	Ulceration of throat	Angina	Croup	Diphtheria	Totals
1851	53	—	—	78	5	38	462	—	636
1852	50	—	—	33	20	43	595	—	641
1853	107	—	—	43	5	16	502	—	673
1854	118	23	—	90	12	10	637	—	772
1855	64	13	—	66	10	12	639	—	804
1856	50	8	4	49	7	14	550	—	682
1857	71	15	10	36	7	21	560	2	622
1858	70	15	2	56	7	11	478	5	644
1859	111	4	3	24	19	58	622	53	894
1860	132	—	6	15	—	37	599	422	1211
1861	101	—	—	25	17	16	460	453	1072
1862	68	—	—	27	7	2	685	594	1383
1863	49	—	—	14	25	64	908	981	2041
1864	18	—	1	4	8	37	754	781	1603
1865	—	—	—	4	24	5	449	584	1016

In regard to the change in nomenclature a quotation from the Registrar General's Report for England and Wales, 1898, is of interest. The conditions described therein undoubtedly apply to a greater or less extent to all other cities and countries. "With regard to the changes that have taken place from time to time in the nomenclature of diphtheria it is important to bear in mind the following points. 1. Diphtheria as a distinct affection had scarcely been recognised in England previous to 1855, in which year this disease was for the first time separated from scarlet fever in the national records of the cause of death. 2. The great diphtheria epidemic of 1858–9 was preceded by a marked increase in the mortality from croup and quinsy, by a still greater increase in the mortality from cynanche maligna, and also by some increase in that from laryngitis. 3. The diphtheria epidemic of 1863 was accompanied by parallel movements in the mortality ascribed to croup and also in that ascribed to cynanche maligna. 4. The incidence of croup in regard both to season and to the ages of death of those attacked is found to have been similar to that of diphtheria. 5. Whereas the deaths attributed to croup in 1861–70 were more numerous by one-third part than those attributed to diphtheria, the deaths attributed to croup in 1896–98 were less than one-sixth part as many as the deaths attributed to diphtheria. These facts taken in conjunction, whilst they afford statistical support to the current medical opinion as to the identity of the two diseases, certainly warrant the assumption that by far the greater number of deaths hitherto attributed to croup have finally been caused by laryngeal diphtheria."

"At the present day the proportion of deaths attributed to 'croup' is relatively so small as to scarcely affect the rate. This is especially true for London where the mortality from 'croup' barely exceeds 3% of that from diphtheria."

This gradual change in nomenclature is well shown by a table giving the annual average rates of mortality during several decennial periods in England and Wales (Registrar General's Report 1898). The figures are per 1,000,000 inhabitants.

TABLE II.

Periods	Diphtheria	Croup	Diphtheria and croup	Laryngitis	Sore throat and quinsy
1861–70	185	246	431	51	14
1871–80	121	168	289	48	10
1881–90	163	144	307	54	24
1891–95	254	70	324	48	21
1896–97	269	43	312	44	19
1898	244	27	271	38	17

This change is particularly well shown in Table I. (1851–65) given on page 575 and by the following continuation also from New York City records.

TABLE III.

	Croup	Diphtheria	Approximate ratio
1858	478	5	96 : 1
1859	622	53	12 : 1
1860	599	422	1·4 : 1
1861	460	453	1 : 1
1862	685	594	1·1 : 1
1863	908	981	·9 : 1
1864	754	781	—
1865	449	534	—
1866	368	435	—
1867	338	251	—
1868	342	276	—
1869	483	328	—
1870	421	308	—
1871	466	238	—
1872	675	446	—
1873	732	1151	0·6 : 1
1874	594	1665	0·5 : 1
1875	758	2329	0·3 : 1
1876	527	1750	0·3 : 1
1877	472	951	0·5 : 1
1878	499	1007	—
1879	522	671	—
1880	910	1390	—
1881	1038	2249	—
1882	729	1525	—
1883	644	1009	—
1884	748	1090	—
1885	855	1325	—
1886	968	1727	—
1887	889	2167	—
1888	639	1914	—
1889	605	1686	—
1890	521	1262	—
1891	609	1361	0·4 : 1
1892	670	1436	0·4 : 1
1893	588	1970	0·3 : 1
1894	511	2359	0·2 : 1
1895	342	1634	0·2 : 1
1896	208	1555	0·1 : 1

Another table (III.) illustrates a similar change in nomenclature in Boston (McCollom, VI. 1905). Taking the yearly percentages of deaths from diphtheria and croup to the total mortality we get the following:

TABLE IV.

	Diphtheria	Croup		Diphtheria	Croup
1879	5·285	2·081	1891	2·914	·501
1880	6·892	2·180	1892	3·684	·596
1881	6·665	2·229	1893	4·064	·597
1882	5·091	1·300	1894	7·092	·529
1883	4·568	1·673	1895	5·190	·582
1884	3·585	1·475	1896	4·435	·481
1885	3·472	1·299	1897	3·684	·403
1886	3·519	1·014	1898	1·562	·138
1887	3·137	·933	1899	2·480	·242
1888	4·609	1·167	1900	4·598	—
1889	5·498	1·160	1901	3·124	—
1890	3·938	·599	1902	1·730	·319

Of the 53,647 deaths from diphtheria and croup reported by the U.S. Census for the five years 1900–04, 33,739 were from the registration states. Dividing these 33,739 into those reported from cities of over 8000 inhabitants and those from the rural districts we find:

Cities over 8000		Rural districts	
Diphtheria	20519	Diphtheria	6728
Croup	3957	Croup	2535

That is to say in the cities 16·2 % of these deaths are called croup, while in the rural districts the percentage is 27·4. Part of this difference is no doubt due to a more accurate diagnosis in the cities, aided as it often is by bacteriological examinations.

The Effect upon Statistics of Bacteriological Examinations in Suspected Cases.

Since Klebs in 1883 demonstrated the existence of a bacillus in the false membrane of diphtheria and Loeffler in 1888 isolated and cultivated this organism and proved its etiological significance, the term "diphtheria" has been limited to exudative or pseudo-membranous inflammation characterised by the presence of diphtheria bacilli. With the increase in our knowledge, we have learned that a picture very similar, clinically and pathologically, to the milder cases of diphtheria can be produced by other bacteria, especially by streptococci. It is disputed by many whether the bacteriological diagnosis adds to or diminishes the number of cases classed as diphtheria. The more severe cases of diphtheria were formerly classed as such and require to be regarded in any statistical study. The less severe cases of true diphtheria were sometimes classed as diphtheria and sometimes not, according to the bias of the physician. As a rule they were not reported as diphtheria. On the other hand, however, there are many cases presenting well-marked membranes on the tonsils and other clinical symptoms of diphtheria, and many exudates complicating scarlet fever which were formerly reported as diphtheria, but which bacteriological diagnosis now proves to be due to other organisms.

In Breslau it has been found that bacteriological examinations fail to confirm the diagnosis in about 10 % of the cases regarded by the physicians as diphtheria, while they show that about 20 % of the cases reported by the physicians as "not diphtheria" are associated with the presence of the diphtheria bacillus. In about 30 % of the cases examined no definite clinical diagnosis is given (Behring, 1901).

Judging from our experience in New York we believe that the culture test probably results in a slight increase in the number of diphtheria cases reported. Mild cases, even if suspected to be diphtheria by the attending physician, would otherwise often go unreported. Cases thus diagnosed as diphtheria are probably more in number than those excluded by the culture test.

This question is discussed in the Annual Report of the Health Department of the City of Boston for 1905, but the authors arrive at a different conclusion.

"In previous reports (1902—1903) calculations have been given to show by two different methods the probable error of the physician in the purely clinical diagnosis of diphtheria. These calculations indicated that at least 38 % of the cases, reported without bacteriological examination as possibly diphtheria, are really not diphtheria. In Boston, from 70 % to 80 % of the total reported cases receive bacteriological confirmation. It seems clear that by means of cultures, in Boston, less cases remain under the designation of diphtheria than if these cases were judged solely on clinical evidence."

While this may be true we believe that the proof afforded by cultures that many slight exudates are truly diphtheria has caused more cases to be officially reported than formerly, and has thus caused a greater proportion of all cases to be included in statistics than in pre-culture times.

The Proportion of Diphtheria Cases sent to Hospitals.

In using hospital statistics for studying diphtheria mortalities it is important to know what proportion of all cases occurring in the district are sent to the hospitals, and what, in general, is the character of these cases. In many cities, owing to an increase in hospital accommodation, to more efficient inspection at the homes, or to other reasons, more cases are sent to the hospitals than in former years. Thus in Berlin between 1884 and 1887 only 26—30% of the total number of cases of diphtheria were sent to hospitals. In 1897 there were 57%, in 1898, 60%, and in 1899, 63% so treated (Gottstein, 1901, p. 605). On the other hand in New York up to the past year there has been no increase of hospital facilities beyond the increase of population, and there has been no appreciable change in the percentage of cases treated in the hospitals.

In making use of hospital statistics it is also necessary to be familiar with the character of the service, *i.e.* the kind of cases which ordinarily come there for treatment. Thus in some European hospitals cases needing tracheotomy are treated in the surgical division, and are not included in statistics published by the medical division. The mortalities in the two divisions therefore differ enormously, and the statistics cannot be compared with those from hospitals in which this division is not made. These differences are well shown by Table VIII. (p. 587) compiled by Gläser (1896) from the records of the Hamburger Allgemeinen Krankenhaus, 1872—1891.

The Influence of the Day of Admission on the Prognosis of Hospital Cases.

This subject is of importance because of its bearing on antitoxin statistics.

There is no doubt that in general the earlier a patient suffering from diphtheria receives suitable care and surroundings, as in a hospital, the better his chance of recovery, irrespective of any specific method of treatment. This fact, which was made use of by those who denied the efficacy of serum treatment, is clearly demonstrated in the following table, published by Gläser (1896) showing the mortality amongst 619 children treated in a hospital without antitoxin.

Here we see that the increase in mortality is quite distinct though somewhat irregular. It is merely necessary, however, to compare these percentages with similarly arranged statistics of cases treated with antitoxin to see at once the great value of early specific treatment (see Table XI., p. 593).

Date of admission	Mortality
2nd day of the disease	19 °/₀
3rd ,, ,,	24 °/₀
4th ,, ,,	38 °/₀
5th ,, ,,	30 °/₀
6th ,, ,,	21 °/₀
7th ,, ,,	41 °/₀

Bing and v. Ellerman (1904, p. 398) have critically studied a large number of cases in order to ascertain the reason of the greater mortality amongst cases received after the 2nd day. They cite Heubner who had caused the prognosis to be written opposite each history on the admission of the patient. The results showed that the patients admitted later were more apt to die, mainly because they were admitted in worse condition than the others. Heubner's results may be tabulated as follows:

Admission on	Percentage of cases with unfavourable prognosis
1st day	6
2nd ,,	8
3rd ,,	14
4th ,,	17
5th ,,	22
6th or 7th days	53
8th and later	69

Bing and v. Ellerman then give their own report on 1356 cases of diphtheria treated in the pre-antitoxin days (1889—1894) in the Blegdams hospital in Copenhagen.

TABLE V.

Day of admission	No. admitted	No. of deaths	Percentage mortality
1	113	38	34
2	494	110	22
3	350	95	27
4	177	68	38
5	125	53	42
6	54	22	41
7	23	13	57
8 and later	20	12	60

They analyse their figures as follows:

"If the mortality is calculated for periods of 48 hours instead of 24 hours the irregularities disappear and one obtains the series 24, 31, 42, 58%. At first sight one would be inclined to agree with Gläser, and others, especially when one remembers that in the last 10 years the mortality of diphtheria has been decreased only from 12% to 4% and that this decrease includes not only the effect of treatment but also any possible spontaneous decrease in virulence which the disease has undergone. If our curve of the pre-antitoxin day is compared with that of Jellineck from the serum period, or with that of Faber (1904), it is noticeable that the rise of the curve is more rapid in the serum period. Furthermore in the serum period one does not see the relatively high mortality on the first day of admission which we found."

The Effect of Heat and Cold on the Prevalence of Diphtheria.

If we glance for a moment at the seasonal and climatic distribution of diphtheria we find that although the disease has prevailed under all circumstances of climate, in the highest as well as in the lowest latitudes, along the coast as well as in the interior, it is particularly in the temperate and colder zones that it effects its greatest ravages. Even allowing for the defective data from equatorial and subtropical countries, we find that in these regions the disease is quite rare.

Hirsch (1886) gives the following data regarding the influence of season and weather, which show that even in temperate climates, where diphtheria is prevalent in cold weather, it is greatly lessened with the approach of summer.

TABLE VI.

		Season			
Country or city	Period	Jan.—Mar.	Apr.—June	July—Sept.	Oct.—Dec.
Sweden	1861–70	31·0	20·9	19·0	29·1
Berlin	1876–83	24·2	21·4	22·1	32·3
Saxony	1873–78	28·7	17·6	17·7	36·0
Hamburg	1873–82	23·9	24·2	21·7	30·2
Göttingen	1878–82	33·5	22·4	19·5	24·5
Schleswig-Holstein	1872–81	29·6	20·0	22·0	28·4
St Petersburg	1878–82	24·7	23·1	20·9	32·3
Frankfurt	1863–83	27·3	24·7	19·6	28·4
Vienna	1863–83	31·3	23·5	15·7	29·5
Philadelphia	1868–75	24·0	21·4	18·5	37·1

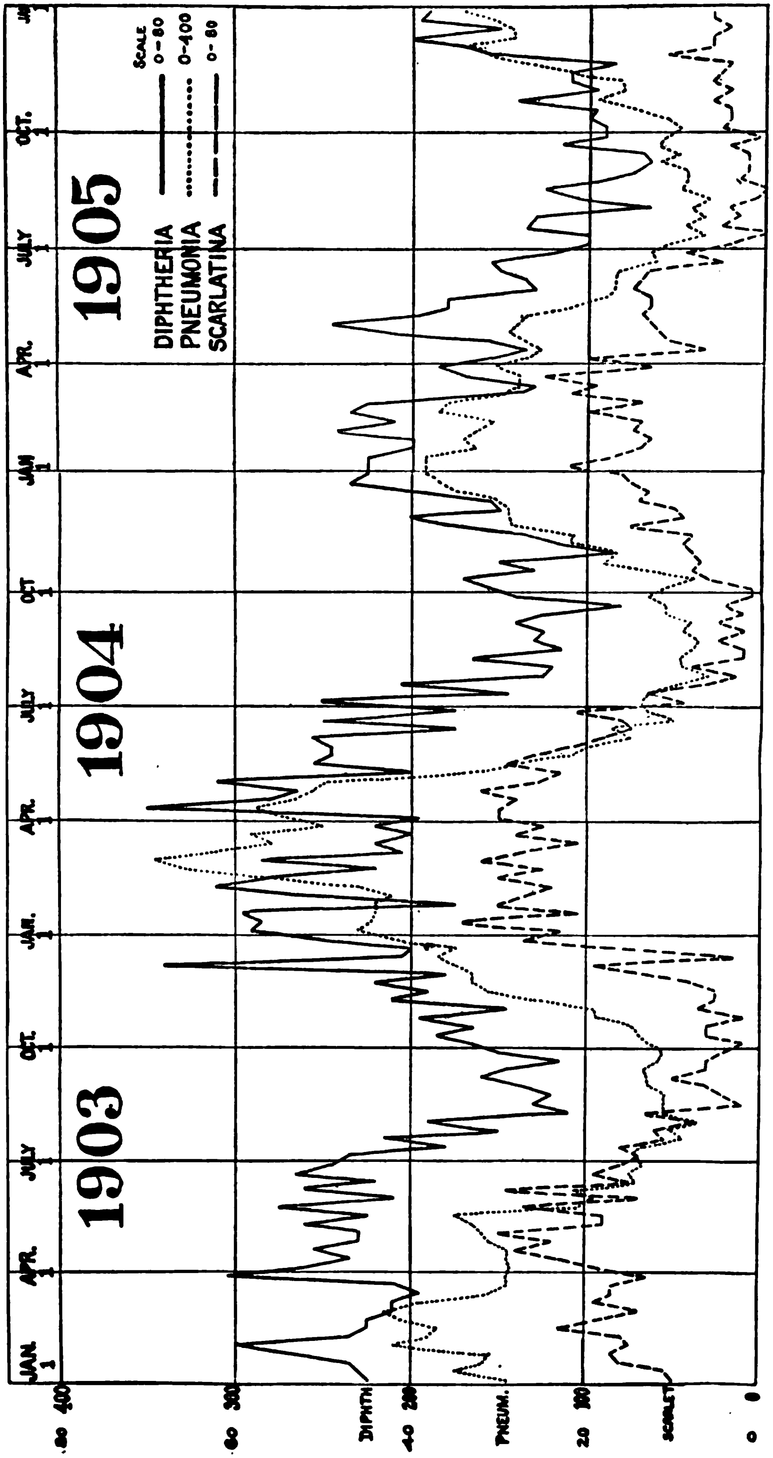


Chart showing the deaths per 100,000 in New York (old city) from Diphtheria, Scarlet Fever and Pneumonia during 1903—1905.

These three-month periods, while they do show that diphtheria is least prevalent in the summer period, do not reflect the influence of weather as well as the Census reports giving the monthly mortality, or the preceding chart arranged by weeks based on the New York returns.

The U.S. Census (Special Reports of the Census Office, Mortality 1900—1904) gives the seasonal distribution of diphtheria and croup, based on 53,647 deaths from diphtheria and croup reported both from the registration and non-registration states during 1900—1904. The figures are per 1000.

January	**112·1**	**July**	**53·6**
February	**92·2**	**August**	**53·2**
March	**83·1**	**September**	**67·8**
April	**75·7**	**October**	**99·8**
May	**70·8**	**November**	**112·9**
June	**65·3**	**December**	**113·5**

The chart on p. 583 shows the deaths per 100,000 in New York (old city) during 1903—1905. Since it is impossible to accurately reflect the meteorological conditions we have plotted on this curve the deaths from pneumonia and bronchitis as well as those from scarlet fever. The pneumonia curve ought to be a fair measure of the effect of weather in producing colds or other diseased conditions of the respiratory tract. Any cold wet weather with slush in the streets is almost certain to be followed by an increase in the number of patients suffering from inflammatory conditions of the respiratory tract and from diphtheria.

The chart shows that diphtheria is at its ebb point during the middle of summer and gradually increases from about the beginning of October. In this rise it follows rather closely the curve of pneumonia and bronchitis which, so far as we can tell, are due to the effect of the weather. Although the pneumonia-bronchitis curve begins to fall about the first week in May, the curve of diphtheria continues at the same level for about a month longer. This is probably due to the fact that the diphtheria bacillus can more easily excite diphtheria, without any aid from atmospheric conditions, than the pneumococcus can cause pneumonia.

In the case of scarlet fever, Seibert of New York has published charts showing that the prevalence of the disease coincides very closely with the sessions of the school. In many years, however, this influence is not so noticeable and, although it is always an important factor, it exerts less effect than the season with its double influence of warmth and outdoor life. How far this holds for diphtheria can be seen from our chart, which also has plotted on it the curve for scarlet fever. While the study of individual cases proves that the spread of diphtheria is aided by school attendance, the curve for large cities proves that here also the weather is an even more important factor. Besides the direct effect of warmth there is the additional effect of ventilation, for in warm weather windows are always open and during the day outdoor life is the rule.

The Influence of Altitude and Soil on the Prevalence of Diphtheria.

The assumption that conditions of the soil have much influence on the development of diphtheria, or on its epidemic diffusion, is one that seems to have no warrant. Hirsch (1886) states that the disease has been as prevalent in elevated as in low-lying places, in mountain regions as on the level ground, on plains as in valleys, on dry as on wet soil, and on porous as on hard rock, and in fact has been equally distributed on the most various geological formations. Nowhere has the independence of the disease on such conditions been more preeminently marked than in England, where the disease on its introduction became quickly and extensively diffused. The observations of Semple, Sanderson, Bottomly and others are thus summed up and endorsed by Ernst Hart (1859).

"Was a connection traced between the localities and its invasions and the marshy ill-drained character of the land, the next season it was found to ravage dry and elevated stations with equal rage. . . . Brighton has not escaped, Scarborough has suffered, it has swept across the marshy lowlands of Essex and the bleak moors of Yorkshire that are swept by the sea breezes; it has seated itself on the banks of the Thames, scaled the romantic heights of North Wales, and has descended into the Cornish Mines."

The same view has been taken by later authorities in England such as Fosbroke, who says "geological position has little, if any, connection with the disease," and the experiences of France, Germany, Holland, Italy, and the United States have been similar.

The Influence of Race.

Whether some races are specially predisposed to take diphtheria, while others enjoy a pronounced immunity from it, cannot be decided with certainty because we possess but scanty information relating to epidemics in localities where the population is mixed.

The Influence of Rainfall.

Newsholme (1898) in an exhaustive study of the effects of increased or decreased rainfall has come to the surprising conclusion that diphtheria only becomes epidemic in years in which rainfall is deficient, and that the largest epidemics occur when three or more years of deficient rainfall follow each other. Our study of his charts does not convince us that he has shown this to be a fact. Some of the places cited could not have been so influenced, for example Manhattan Island with its rocky formation where the amount of rainfall would exert little effect on the ground water, one of his supposed factors. In cold weather dampness predisposes to respiratory troubles and so to diphtheria, especially of the laryngeal type.

The Influence of Occupation.

Diphtheria being especially a disease of childhood is not greatly influenced by occupation. Crowding and lack of ventilation predispose to it.

THE MORTALITY AND PREVALENCE OF DIPHTHERIA AT DIFFERENT AGES.

Diphtheria is essentially a children's disease, a fact emphasised by all writers. This does not mean that only children are attacked, for the disease is certainly not rare among adults.

During the first few months of life there is only a slight disposition towards the disease. Towards the end of the first year, however, this disposition grows rapidly, increasing still more rapidly during the second year, so that from that age until about the close of the fifth year the liability to the disease is at its height. Subsequently the disposition drops, at first slowly, then more rapidly, so that from about the 14th to the 15th year the disposition is again very slight.

The following figures show the age incidence of 2711 cases admitted to Baginsky's (1898) children's hospital in Berlin between 1890 and 1897.

TABLE VII.

Age	No. of cases	Percentage of total cases	No. of deaths	Death rate per 100 admitted	Death rate at each age period
0—6 months	15	0·55 %	4	0·15	26·6
6 mths.—1 year	69	2·50	36	1·32	52·2
1—2 years	227	8·30	110	4·05	48·4
2—3	317	11·60	119	4·30	37·5
3—4	354	13·05	121	4·40	34·2
4—5	337	12·40	84	3·09	24·9
5—6	264	9·70	61	2·20	23·1
6—7	280	10·30	61	2·20	21·8
7—8	209	7·70	26	0·95	12·4
8—9	175	6·40	23	0·84	13·1
9—10	146	5·30	19	0·70	13·0
10—11	101	3·70	9	0·33	8·9
11—12	80	2·90	8	0·29	10·0
12—13	65	2·02	7	0·25	10·8
13—14	72	2·60	6	0·22	8·3
	2711		694		25·6 % Av.

These figures, being from a children's hospital, do not reflect the age distribution as well as those from a hospital treating adults also, such as the following for 4127 cases, compiled by Gläser (1896) from the Hamburg Allgemeinen Krankenhaus 1872-91.

TABLE VIII.

	Medical Division			Surgical Division almost exclusively tracheotomies			Entire Hospital			Proportion of each age group admitted to total admissions (4127) per cent.
Age	Cured	Died	Mortality %	Cured	Died	Mortality %	Cured	Died	Mortality %	
Under 1 year	5	17	77	3	34	91	8	51	86	1·4
1—2	38	68	64	34	179	84	72	247	77	7·5
2	50	53	51	68	152	69	118	205	63	7·6
3	80	80	50	126	185	59	206	265	56	11·2
4	108	76	41	127	138	52	235	214	47	11·0
										Total under 5, 38·7 %
5	89	45	33	90	84	48	179	129	41	7·6
6	113	47	29	47	64	57	160	119	40	6·7
7	119	43	26·5	37	44	54	156	87	35	6·0
8	86	31	26·4	13	19	59	99	50	33	3·4
9	66	17	20·4	7	6	46	73	23	23	2·2
10	78	20	20·4	7	9	56	85	29	25	2·6
										Between 5 and 10, 28·5 %
10—12	125	3	2·3	2	3	60	127	6	4·5	3·0
12—15	144	5	3·3	3	9	75	147	14	8·6	3·5
15—20	478	13	2·6	—	—	—	—	—	—	11·5
20—30	450	6	1·3	—	—	—	—	—	—	11·1
30—40	64	2	3·0	—	—	—	—	—	—	1·6
40—50	14	2	12·5	—	—	—	—	—	—	·5
50—60	3	—	—	—	—	—	—	—	—	
60—70	3	—	—	—	—	—	—	—	—	

The U.S. Census (Special Report 1900–04) gives the age distribution of *deaths* from diphtheria and croup in a very large number of cases.

Sex	
Male	27299
Female	26348
	53647

Age	Number	Percentage
Under 1 year	3736	7
1 year	8001	15
2 years	7990	14½
3 ,,	7207	13½
4 ,,	5989	11
Under 5 years	32923	61
5—9 years	14818	27
10—14 ,,	3191	6
15 years and over	2628	5
Unknown	87	—
Total	53647	

Incidentally this table shows that the conditions of life in a large crowded city like New York are especially hard on children under 5 years of age, for in New York over 80 % of the diphtheria and croup deaths occurred in children under 5, while in the above table only 61 % of these deaths occurred in that age period.

The following table shows the age distribution of the *fatal cases* in New York City for 10 years.

TABLE IX. *Deaths from diphtheria during* 1891–1900 *in the city of New York. Age Distribution.*

Age	Female	Male	Total	Percentage of total
Under 1 year	745	920	1665	9·2
Between 1 and 2 years	1998	2265	4263	23·6
,, 2 and 3 ,,	1872	1945	3817	21·2
,, 3 and 4 ,,	1412	1488	2900	16·1
,, 4 and 5 ,,	960	948	1908	10·6
Total under 5	6987	7566	14553	80·6
Between 5 and 10 years	1681	1371	3052	17·0
,, 10 and 15 ,,	131	110	241	1·3
,, 15 and 20 ,,	31	29	60	1·7
,, 20 and 25 ,,	36	22	58	
,, 25 and 35 ,,	49	46	95	
,, 35 and 45 ,,	19	22	41	
,, 45 and 55 ,,	13	16	29	
,, 55 and 65 ,,	9	7	16	
,, 65 and 75 ,,	4	4	8	
,, 75 and 85 ,,	0	—	—	
85 or over	1	—	1	
Total			18003	

According to the Registrar General's Report (*Lancet*, 1878) the age distribution amongst nearly 70,000 *cases* in England was as follows:

Under 1 year	9·0 °/₀
From 1—5 years	45·0
„ 5—10 „	26·0
„ 10—15 „	9·0
„ 15—25 „	5·0
„ 25—45 „	3·5
45 years and over	2·5

The youngest case of which we can find any record is that of a child 9 days old, reported by Jacobi. Forest (1905) has recently reported three cases in young infants 19 days, 3 weeks, and 11 weeks old respectively. We have met with two severe pharyngeal cases at an early age, 17 and 21 days respectively.

Sex seems to play no part in this disease, boys and girls being attacked in about equal numbers. Of the 2711 (p. 587) cases previously mentioned, 1311 were boys and 1400 girls. The mortality among the former was 27·9 %, among the latter 23·2 %.

INFECTIVITY AND VIRULENCE.

There is considerable variation in the mortality at different times and in different places. This fact is particularly important in considering the influence of antitoxin on this disease.

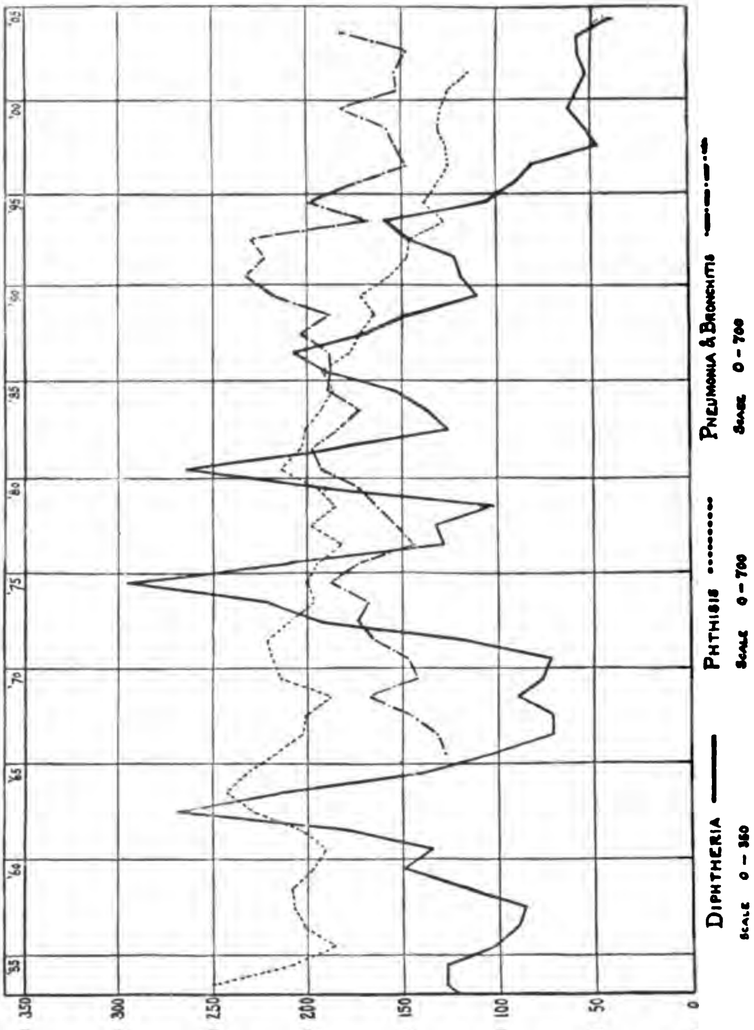
First of all it is necessary to appreciate clearly that poison production and infectivity are two entirely independent properties. Thus a marked capacity for producing a severe attack of a disease may be associated with a very low degree of infectivity, while the presence of great power of infectivity in an organism or mixture of associated organisms does not imply that the average type of disease produced will be necessarily severe.

J. Eröss (1904, p. 593) finds in studying the statistics from a large number of Hungarian cities that the case mortality in diphtheria is greatest at the height of the epidemic, and that as soon as the disease loses its epidemic character, the case mortality drops to a low level. Newsholme (1898) proves that this is not always the case, and gives a number of interesting tables, which show the reverse behaviour. Brownlee (1905) in an excellent paper on this subject states that in England the disease is as a rule most fatal in towns where it is least prevalent. We think that in some instances this is due to the fact that in years when the disease is not epidemic the proportion of laryngeal cases is greater than usual.

THE CHARACTERISTICS OF EPIDEMICS.

Statistical studies in order to be of value must embrace a sufficient period of years and a sufficiently large number of cases. This is particularly true of diphtheria, a disease which recurs in epidemic cycles of varying length, and is well shown by the following curve, which is perhaps as accurate as any which can be found.

Deaths per 100,000 in New York (old city) between 1854—1905.



This chart requires further explanation. For the period from 1853–55 the deaths are all those given in Table I., p. 575, while from 1866 the figures include all the deaths due to diphtheria and croup. During the early part of these years the deaths are undoubtedly understated, for we know that only from two-thirds to three-quarters of all deaths were really reported. For the past fifty-five years, however, the figures are probably very near the actual conditions.

In the case of this city, then, we see that there are irregular epidemic recurrences at intervals varying from about five to ten years. The importance of bearing in mind these cyclical recurrences will be discussed in connection with antitoxin treatment (p. 599).

THE RESULTS OF ANTITOXIN TREATMENT.

Comparison of Case Mortalities.

Diphtheria antitoxin was introduced more than ten years ago, consequently sufficient time has elapsed to enable us to study the effect this treatment has had on the mortality from diphtheria.

An examination of the literature shows that such studies have been made a number of times, and that many of them are exceedingly valuable. Nevertheless objections have been made, particularly by opponents of the serum treatment, that such statistics are open to grave sources of error, and that the apparent improvement is only temporary. Instead of this contention receiving support as the antitoxin treatment extends over a longer period and becomes more general, we find that in spite of temporary fluctuations the trend is always toward a lower mortality.

In the early period of antitoxin administration (in 1896) the American Pediatric Society collected reports on 5794 cases of diphtheria treated with antitoxin. These showed an average mortality of 12·3 %.

Guerard (1896) collected reports of 9893 cases treated with the serum, with an average mortality of 18·3 %. Of these cases, 7277 were treated in 53 hospitals, and in them the mortality was 20 %. The average mortality in the same hospitals had previously been 44·3 %.

Ernst reported on 1156 cases treated by the serum in the City of Boston, amongst whom 165 deaths occurred giving a mortality of 14·2 %. McCollom's (1905) report on the diphtheria wards of the Boston City Hospital shows even better results. Amongst 844 cases treated by the serum there were 96 deaths, a mortality of 11%. The previous mortality in the same institution without serum was 40%.

Siegert (1900) tabulated the results of the cases treated in 23 hospitals for children in Europe from 1890 to 1898 inclusive. Omitting 1894, the transition year, his figures are as follows:

Period	Cases treated	Deaths	Mortality
Before serum 1890–1893	16585	6889	41·3
After serum 1895–1898	20181	3309	16·4

The following table from the Annual Report of the Metropolitan Asylums Board 1904 shows a marked reduction in the mortality from diphtheria since 1889.

TABLE X. *Cases treated in the Hospitals of the Metropolitan Asylums Board, London.*

	Total cases admitted	Number of deaths	Percentage mortality	
1889	722	275	40·7	
1890	942	316	33·5	
1891	1312	397	30·6	
1892	2009	583	29·3	
1893	2848	865	30·4	
1894	3666	1035	29·3	Antitoxin first used in the autumn.
1895	3635	820	22·8	
1896	4508	948	21·2	
1897	5673	987	17·6	
1898	6566	991	15·3	
1899	8676	1182	13·9	
1900	7873	988	12·2	
1901	7622	849	11·1	
1902	6520	739	11·0	
1903	5072	504	9·7	
1904	4687	469	10·0	

Reuter in 1901 published statistics from the Eppendorf Hospital in Hamburg. His curve from 1890 to 1900 shows a marked drop after the introduction of serum treatment. Cohn in 1904 published excellent statistics on 1000 cases from the Moabit Hospital in Berlin. They were all antitoxin cases, and the mortality was 13·6%. Only those cases which proved to be diphtheria clinically and bacteriologically are included. Zucker's figures published in 1905 are reproduced later in the form of a curve (p. 595). The difference between the patients receiving and not receiving antitoxin is very striking. In the *Statistische Monatsschrift* 1902 (quoted by Eröss, 1904, p. 593) we find that in Austria during 1896–1899, 14·5 to 16·1 % of the patients treated with serum died, while 36·4 to 39·8 % of those not treated died, *i.e.* 2½ times as many.

Bokay (1904) says that in 1891 amongst 2952 cases of diphtheria in Buda-Pesth 914 died (32·5 %), while in the year 1903 out of 2293 cases only 309 died (13·5 %). He says by merely placing these two years side by side there is enough statistical material to show the great value of serum treatment.

The above figures exemplify the "case-mortality method." This method is open to several objections, the chief amongst them being the fact that the average severity of the cases admitted to the hospitals may have differed in the two periods. This is undoubtedly true to some extent, as for instance in Boston, where a new and better hospital has been built and a larger percentage of cases seek admission.

The Influence of the Time of Administration on the Value of the Antitoxin Treatment.

In view of the scepticism of many as to the value of statistics based on the case mortalities before and since the introduction of antitoxin, a number of observers have sought to show the value of serum treatment by tabulating the days on which such treatment was begun and the corresponding mortality. Two examples of this will suffice. Faber (1904) tabulated the results in 3167 cases of diphtheria treated in the Blegdams Hospital, Copenhagen. He excludes cases complicated with scarlet fever, whooping cough and other diseases unless the patients were convalescent from them.

TABLE XI.

Commencement of serum treatment	No. of patients	No. of deaths	Percentage mortality	Calculated No. of deaths according to the entire mortality of the group (11·5 %)	Difference between actual and calculated mortality
1st day	99	7	7·1	11	– 4
2nd ,,	641	48	7·5	74	– 26
3rd ,,	763	69	9·0	88	– 19
4th ,,	555	63	11·4	64	– 1
5th ,,	334	52	15·6	38	+ 14
6th ,,	171	29	17·0	20	+ 9
7th ,,	80	17	21·3	9	+ 8
Later than 7th	196	39	19·9	23	+ 16
Unknown	298	35	—	—	—
Total	3137	359	Average 11·5		

Cohn (1904) gives the following figures for 1000 cases of diphtheria treated in the Moabit Hospital, Berlin:

	Cases	Deaths		Percentage mortality
Treatment begun on 1st day	78	1	=	1·3
,, ,, ,, 2nd ,,	361	40	=	11·1
,, ,, ,, 3rd ,,	284	30	=	10·5
,, ,, ,, 4th ,,	101	25	=	24·7
Later or undetermined	176	40	=	22·7

Similar statistics have been published by Heubner, Aaser, Frank, and Jellineck. The mortality increased from 4 or 5 % on the first day to 20 % or more when the serum was administered later in the disease.

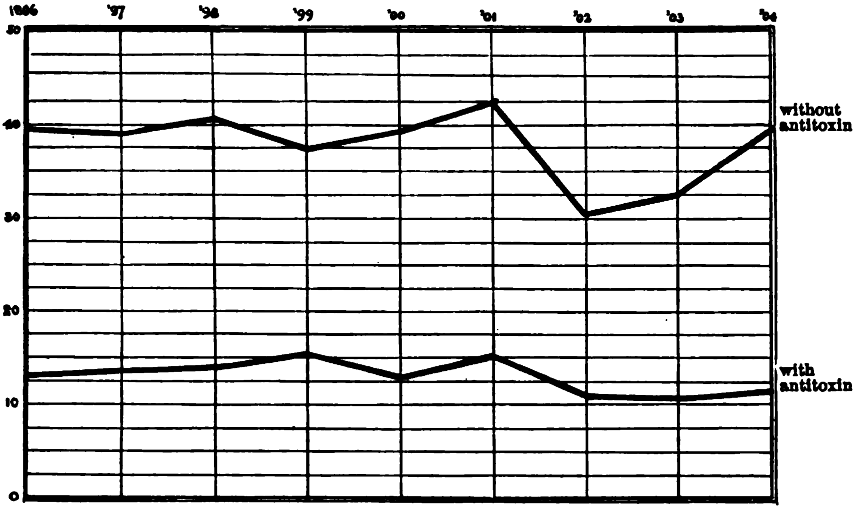
This method is open to at least one objection. The children brought to the hospital on the first few days of the disease are on the whole in better condition than those brought later. Many of the latter class are brought to the hospital as a last resort, or because operative interference is needed. If the non-antitoxin statistics previously quoted (p. 580) are compared with these it is evident that the actual mortality of the antitoxin cases admitted during the first two days is much smaller than of the patients to whom antitoxin is not given.

Regarding a possible Decline in the Virulence of the Disease in Recent Times.

It has been urged, especially by opponents of serum therapy, that apart from the use of diphtheria antitoxin the disease itself is becoming milder. Careful study of the subject fails to bear out this contention, for among those who do not receive antitoxin the mortality at the present day is still as high as ever. This question has recently been studied, especially by Zucker (1905), who found among the rural population in his province abundant statistics on cases not treated with antitoxin. Thus in Steiermark at the highest estimate only two-thirds of the cases received antitoxin. The total number of cases which did not receive antitoxin (since the introduction of the serum treatment) was 12,000. His chart which is here reproduced shows very well the difference in mortality between those cases receiving antitoxin and those not receiving it.

Zucker (1905) gives analogous figures for non-antitoxin cases from the statistics of Graz. In these the case mortality was 21·7 % in the quinquennium 1894–98 and 22·6 % in the quinquennium 1899–1903.

Diphtheria case mortality in Steiermark, 1896—1904 (*after Zucker*).



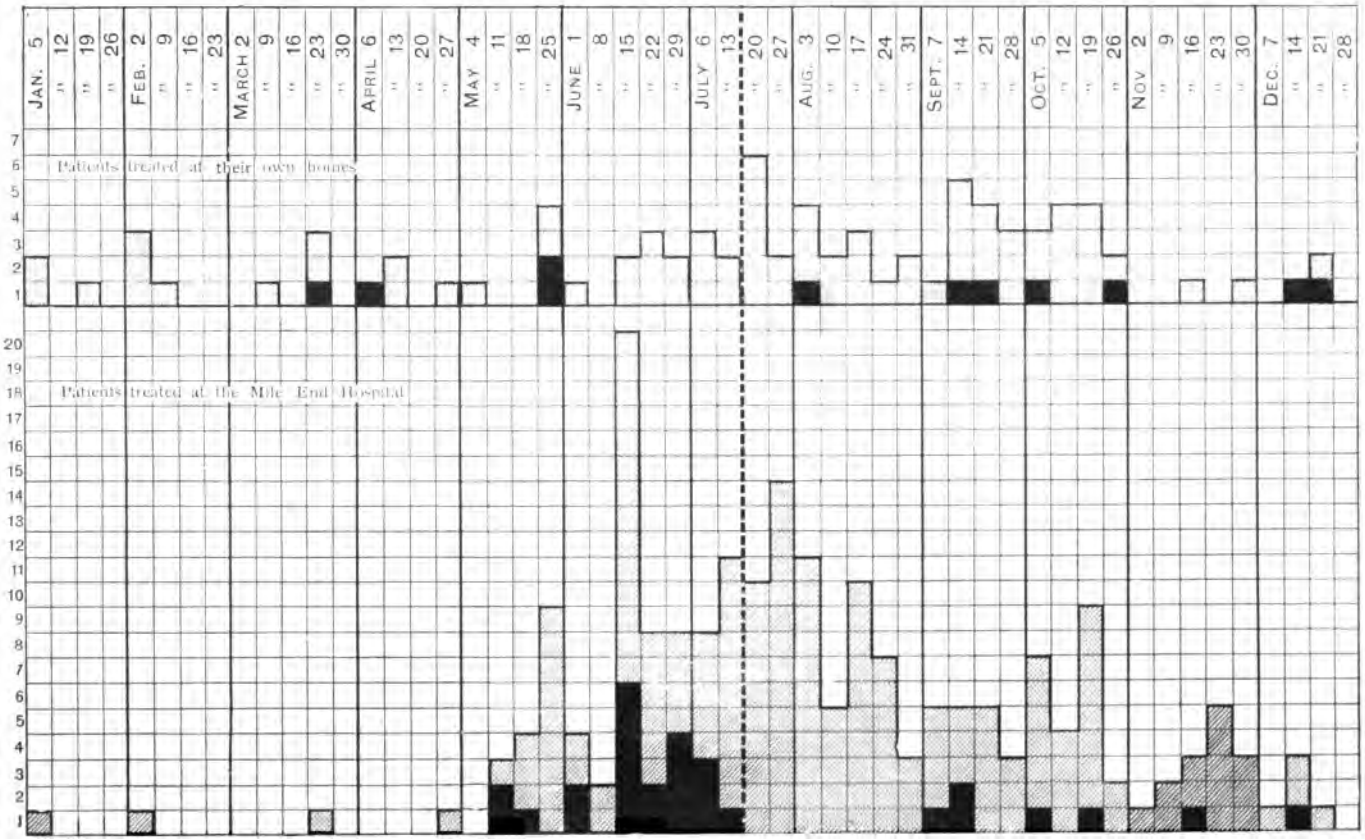
The effect of the antitoxin treatment is also well brought out by Cobbett and Graham-Smith's (1901) observations on the outbreak of diphtheria in Colchester during 1901. During this year 286 cases of diphtheria were notified, and of these 200 were treated in the Infectious Diseases Hospital. "Prior to July 16 antitoxin was not given as a routine treatment on the admission of the patient to the hospital, but appears to have been administered to a few serious cases only, and to some others who developed alarming symptoms later. During this period reliance was placed on antiseptic sprays to destroy the bacilli in the throat. After July 16, antitoxin was administered in accordance with a resolution of the Sanitary Committee to every case, as soon as possible after admission, unless this had been previously done, and no efforts were made to destroy the bacilli in the throat.

During the first of these periods 89 patients were treated in the hospital and 21 deaths occurred. The case mortality was accordingly 23·6 per cent.

During the second period 113 cases were treated, and eight deaths occurred. The case mortality was therefore 7·8 per cent.

But of the 89 patients treated during the first period 15 were known to have had antitoxin administered before admission; and among these there were no deaths. Moreover, during the second period, two patients were treated without antitoxin by their private

Weekly notifications of Patients suffering from Diphtheria at Colchester during 1901.



▨ **represents a notified case of Diphtheria.** ■ **represents a notified case of Diphtheria which terminated fatally.**

July 16th, systematic use of Antitoxin introduced into the hospital.

medical attendants, and one of them died. Therefore, if we wish to compare the mortality among the patients treated at the hospital on the system which prevailed during the first period with that among the patients treated before or immediately after admission with antitoxin we must make a correction for these persons. When this has been done we find that the mortality was 28·9 and 5·6 per cent respectively.

The fall in the case mortality from 23·6 to 7·8 which occurred in the hospital on the introduction of the systematic use of antitoxin, or if we prefer to take the corrected figures, from 28·9 to 5·6 per cent., affords a striking example, if any were needed, of the value of this remedy.

The fall in the case mortality at the hospital has been ascribed to a gradual diminution in the severity of the disease. The weekly admissions and deaths from diphtheria in the hospital, however, show that the change in the mortality was abrupt, and that the introduction of the new treatment was followed by a succession of sixty cases without a single death.

But the most convincing evidence that the fall in the case mortality of patients treated in the hospital was due to the use of antitoxin, and not to a decline in the severity of the disease, is shown by the concurrent mortality of patients treated in their own homes; for upon the case mortality of these patients the resolution could have had little or no influence.

Amongst these latter the case mortality was 10·8 per cent. before the regular use of antitoxin was commenced (37 cases) and 13·3 per cent. after (46 cases). It is quite evident then that the severity of the disease did not decline, and it may fairly be concluded that the resolution of the Sanitary Committee resulted in the saving of a considerable number of lives."

OPERATIVE CASES.

A comparison of the mortality in the operative cases is of particular interest, since these are always suffering from a severe type of diphtheria.

The following figures are compiled from Lovett and Munro (1887), and McCollom's (1905) papers and from the New York official records.

TABLE XII.

Operative Cases	*Treated without antitoxin*		
	Total cases	Died	Percentage mortality
German Authors	5795	3944	69
,, Hospitals	3063	2124	70
British Authors	433	295	69
French Authors	9242	6834	76
American Authors—Private Practice ...	5625	3848	68
Various Countries	1993	1336	68
Boston City Hospital (23 years)	—	—	71
	Treated with antitoxin		
Welch (1894)—European Hospitals ...	342	112	29·8
Amer. Pediatric Soc. Report—Private Practice	533	138	25·9
McCollom—Hospital Cases—Boston ...	1553	683	44·0
New York Health Dept. as below	1660	723	43·5
Tenement Service, 1895–7 144 cases, 56 deaths			
,, ,, 1902–4 133 ,, 39 ,,			
Diphtheria Hospital, 1901–5 1341 ,, 614 ,,			
Outside Physicians, 1895–6 42 ,, 14 ,,			

Roux gives the mortality in operative cases as 46 °/₀ as compared to 76 °/₀ in pre-antitoxin times. With Ganghofner (1899) it is 13·6 as compared to 59·8—78 °/₀. According to the Hungarian Health Authorities it is 32·3 °/₀ as compared to 50—70 °/₀ formerly. Cohn (1904) reports 67 deaths out of 259 cases operated on for laryngeal stenosis = 25·9 °/₀ mortality. Formerly the mortality was over 70 °/₀.

But not only are the results in operative cases much more favourable since the introduction of antitoxin, but it has also been noted by most authors that fewer laryngeal cases need to be operated on than formerly.

STATISTICS ON ALTERNATE CASES.

An ideal method of obtaining statistics was one made use of by Fibinger (cited by Faber, 1904). During one period alternate cases were treated with and without antitoxin.

Amongst 238 cases treated with antitoxin 8 died (mortality 3 %).

Amongst 245 cases treated without antitoxin 30 died (mortality 12 %).

This method, however, for obvious reasons cannot now be made use of. We once made a similar test at the Willard Parker Hospital, but the difference in the behaviour of the cases was so greatly in favour of those treated with antitoxin that the test was stopped and all cases put on antitoxin.

THE ABSOLUTE MORTALITIES DURING A LONG SERIES OF YEARS IN LARGE CITIES AS AN INDEX OF THE VALUE OF ANTITOXIN TREATMENT.

Of all methods at present available perhaps the least open to error is a comparison of the absolute number of deaths per 100,000 before and after the introduction of antitoxin.

Such statistics were published by Bayeux (1899, cited by Müller 1902) and by Erich Müller (1902). The former's statistics, beginning in 1888 and ending in 1897, dealt with French cities having a population of over 20,000 inhabitants. The aggregate population per year was over eight millions, and the mortality during the three antitoxin years was only about one-third the mortality of the former period.

Müller's figures were obtained from German cities having over 40,000 inhabitants; the population amounting to 8½ million people in 1889 and over 12 millions in 1900. His results are summarised in the following table.

	Pre-antitoxin period						Antitoxin period					
Year:—	1889	1890	1891	1892	1893	1894	1895	1896	1897	1898	1899	1900
Deaths per million inhabitants	108	102	81	93	118	96	51	39	32	30	28	25
	Average 100						Average 34					

To be of value statistics of this kind must cover a long period of years. While this, of course, is true for all kinds of statistics it is particularly important in diphtheria in which mortality figures move up and down in irregular waves. These irregularities, however, only become apparent when a considerable number of years is taken into account. For example, in the six years ending 1882 the average of deaths per 100,000 from diphtheria and croup was always above 140 in Baltimore, and reached 200 or over in three of these years. In the seven years following the mortality fell sharply and continuously until it reached its lowest point in 1889, when it was 52 per 100,000, and yet no difference in treatment occurred in 1882.

Care must therefore be taken to secure readings which do not merely constitute part of an epidemic, unless due allowance be made for this fact. In order to distinguish epidemics it is necessary to know the average number of deaths for many years. Lack of this knowledge was the weak point in a number of earlier statistical studies and was made use of by the opponents of serum therapy (de Maurans, Kassowitz). The former investigator, for example, published the statistics of numerous cities in which the drop in mortality had either already begun prior to the use of antitoxin, or in which since that period the mortality had actually increased. It will suffice here to say that, while this was undoubtedly true, the aggregate number of deaths in the pre-antitoxin years, in all the cities cited by de Maurans, was much greater than in the antitoxin years, and as Dessaux pointed out, in the pre-antitoxin years the minimum annual number of deaths in de Maurans' cities was 7858, while the maximum number in the antitoxin years was 6598. Further, statistics of this kind ought to be taken mainly from the large cities, for reports of deaths are usually indifferently kept in the rural districts. Thus in some States the cause of death used often to be certified to the health authorities by the town supervisor, so that "sore inside," "chronic," and "running sores" were occasionally given as causes of death.

In compiling the following statistics therefore we have only taken cities having over 125,000 inhabitants, and in which the registration of deaths has long been efficient. Apart from these two points the cities were taken at random, some from the United States, some from Great Britain, and the remainder from the continent of Europe. The figures are in all cases from the official records, and are the combined "diphtheria" and "croup" figures. In order to overcome the fluctuations commonly seen in diphtheria mortalities we have gone back to 1878, *i.e.* 15 years prior to the introduction of antitoxin, and have carried our figures up to the 31st of December, 1905, *i.e.* 10 years after the use of antitoxin. We believe that the evidence thus obtained of the great value of diphtheria antitoxin is overwhelming, especially when added to the French and German statistics already referred to.

TABLE XIII. *New York City (Present Boroughs, Manhattan and Bronx).*

Year	Population	Deaths from diphtheria and croup	Deaths per 100,000
1851	538490	636	118
1852	560607	641	114
1853	583632	673	115
1854	607603	772	127
1855	632559	804	127
1856	664980	682	102
1857	699062	622	90
1858	734892	644	87
1859	772558	894	116
1860	812154	1211	140
1861	794905	1072	135
1862	778023	1383	179
1863	761500	2041	268
1864	745327	1603	215
1865	729498	1016	140
1866	767979	803	104
1867	808489	585	72
1868	851137	616	72
1869	896034	813	90
1870	943300	729	77
1871	955921	704	73
1872	968710	1121	116
1873	981676	1883	192
1874	1030607	2259	219
1875	1044896	3087	295
1876	1075532	2277	211
1877	1107597	1422	128
1878	1140617	1506	132
1879	1209268	1193	101
1880	1174621	2300	190
1881	1244511	3287	264
1882	1280857	2254	184
1883	1318264	1653	125
1884	1356764	1838	136
1885	1396388	2180	158
1886	1437170	2695	188
1887	1479143	3056	206
1888	1522341	2553	167
1889	1566801	2291	146
1890	1612559	1783	110
1891	1659654	1970	118
1892	1708124	2105	123
1893	1758010	2558	145
1894	1809353	2870	158
1895	1873201	1976	105
1896	1906139	1763	91
1897	1940553	1590	81
1898	1976572	923	46
1899	2014330	1085	53
1900	2055714	1276	62
1901	2118209	1227	58
1902	2182826	1142	53
1903	2249680	1270	56
1904	2318831	1270	57
1905	2390382	860	38

The Antitoxin Laboratory established in the autumn of 1904. Free distribution to the poor begun early in 1895.

The deaths classed in this table as due to diphtheria are only approximately correct. They include all the deaths during the period 1851—1865, which were returned under any of the following heads, Angina, Croup, Diphtheria, Inflammation of throat, Inflammation of tonsils, Quinsy, Sprue, Ulceration of throat. It is estimated that prior to 1851 not more than two-thirds of the deaths were registered. Since then conditions have gradually improved. It must also be remembered that scarlet fever and diphtheria were often confounded in these earlier days.

TABLE XIV.

	Baltimore.					Philadelphia.					
Year	Population	Deaths from diphtheria and membranous croup	Croup	Deaths from diphtheria and croup	Deaths per 100,000	Population	Deaths from diphtheria	Deaths from croup		Total deaths	Deaths per 100,000
								Membranous	Simple		
1877	311275	455	157	612	197						
1878	318182	302	149	452	142	825000	464	388		854	103
1879	325139	298	186	484	150	835000	321	291		612	73
1880	332313	293	173	466	140	846980	323	303		626	74
1881	339649	639	242	881	260	868000	457	317		774	90
1882	347142	707	222	929	265	886539	933	466		1399	158
1883	354832	591	201	792	143	907041	1006	500		1506	166
1884	362668	343	127	370	102	927995	680	589		1269	135
1885	370696	252	148	300	81	949432	600	753		1353	143
1886	378903	190	128	318	84	971363	411	650		1061	109
1887	387300	149	153	302	78	993801	416	442		858	86
1888	395999	118	98	216	54	1016758	350	273		623	61
1889	404498	155	53	208	51	1040245	375	352		727	70
1890	413671	274	45	219	77	1046964	528	415		943	90
1891	436917	350	44	294	92	1069264	918	444		1362	129
1892	440163	381	47	428	98	1092168	1435	272		1707	156
1893	453409	135	25	310	69	1115562	916	273	79	1238	110
1894	466655	198	33	231	50	1139457	1047	348	56	1451	128
1895	479907	265	45	310	65	1163864	1020	329	49	1398	120
1896	493147	249	32	281	57	1168793	862	293	46	1201	103
1897	500000 estmd.	347	13	360	72	1214256	1231	243	40	1514	125
1898	505000 ,,	362	50	412	81	1240266	998	156	21	1175	94
1899	509000 ,,	312	—	312	61	1266832	849	144	21	1014	80
1900	513000 ,,	267	12	279	54	1293697	898	144	25	1063	82
1901	518000 ,,	171	—	171	32	1321408	525	118	18	661	50
1902	525000 ,,	130	—	130	25	1349712	435	80	16	531	40
1903	533000 ,,	160	—	160	30	1378624	521	87	22	631	46
1904	541000 ,,	115	—	115	21	1408154	458	83	7	548	38
1905	550000 ,,	112	—	112	20	1438318	401	51	10	462	32

Philadelphia has distributed antitoxin free of charge for use among the poor since 1896. For several years after this, however, its use was not very general. Since about 1900, however, its use has extended as is shown by the following figures. There were supplied: 1896, 900 doses; 1897, 2334 doses; 1898, 3367 doses; 1899, 2976 doses; 1900, 5233 doses; 1901, 5374 doses; 1902, 5771 doses; 1903, 7342 doses; 1904, 10298 doses.

TABLE XV.

Year	*Berlin.* Population	*Berlin.* Deaths from diphtheria and croup	*Berlin.* Deaths per 100,000	*Boston*.* Population	*Boston*.* Deaths from diphtheria and croup	*Boston*.* Deaths per 100,000	*Breslau.* Population	*Breslau.* Deaths from diphtheria and croup	*Breslau.* Deaths per 100,000
1876	—	—	—	346004	720	208	—	—	—
1877	—	—	—	350138	471	134	—	—	—
1878	1033632	1446	140	354322	569	160	267000	106	40
1879	1065440	1355	127	358554	545	152	270000	105	39
1880	1107100	1422	129	362839	774	213	272000	135	50
1881	1138700	1778	156	368190	802	217	275000	158	57
1882	1174293	2134	181	373620	575	153	280000	163	60
1883	1207114	2932	243	379129	608	160	287000	323	113
1884	1225065	2640	215	384720	487	126	295000	230	81
1885	1299207	2007	154	390393	459	117	297413	218	73
1886	1337798	1688	126	401374	423	105	302759	280	92
1887	1376389	1404	112	412663	410	99	308105	497	125
1888	1414980	1100	79	424274	589	138	313451	495	160
1889	1453571	1284	88	436208	683	156	318797	382	120
1890	1492162	1591	107	448477	462	103	324143	362	111
1891	1609415	1075	66	457772	285	62	389318	323	95
1892	1662237	1414	85	467270	481	102	346442	357	74
1893	1714938	1643	95	476270	546	114	353331	409	116
1894	1767639	1430	81	486830	878	180	360660	314	87
1895	1669138	1000	59	501083	588	117	370038	242	65
1896	1695726	569	33	516305	516	98	378089	125	33
1897	1708499	547	32	528912	411	77	385108	98	25
1898	1728201	664	38	541827	170	31	398415	93	24
1899	1747903	655	37	555057	277	49	405362	94	23
1900	1864203	563	30	560892	537	95	412959	49	12
1901	1913528	513	26	573579	353	61	427883	71	17
1902	1955827	231	11	586533	225	38	436618	87	20
1903	1998146	245	12	600929	214	35	445403	96	21
1904	2040455	354	17	588320	206	35	454188	85	18
1905	2026000	310	15	595380	132	22	465000	92	20

* **Remarks:** Antitoxin Laboratory established 1895. First serum issued Sept. 1, 1895. Free distribution of antitoxin to the poor since 1896. It was quite generally used from the first. At present about 60 % of all cases of diphtheria are treated in the hospital.

TABLE XVI.

	*Brooklyn**.			*Cologne.*			*Dresden.*		
Year	Population	Deaths from diphtheria and croup	Deaths per 100,000	Population	Deaths from diphtheria and croup	Deaths per 100,000	Population	Deaths from diphtheria and croup	Deaths per 100,000
1878	536561	861	162	138836	45	40	210377	217	103
1879	551397	939	170	140104	55	39	215400	159	74
1880	566663	1538	270	144735	82	57	220216	284	130
1881	585017	1607	275	146000	78	53	223100	381	170
1882	603926	965	160	146767	66	45	227250	534	235
1883	623422	727	116	148640	55	37	233600	495	212
1884	643487	665	103	151500	29	19	236000	467	200
1885	664126	832	125	160015	70	44	243980	342	140
1886	707092	1185	167	163341	87	53	249034	416	167
1887	730712	1453	200	166667	79	48	254088	330	130
1888	755145	1265	164	169993	99	57	259142	268	104
1889	856321	1467	180	261105	89	38	264198	268	101
1890	894024	1283	152	267152	173	65	269250	253	94
1891	933398	1180	135	286230	255	90	297585	256	91
1892	970046	1137	126	295059	359	120	286160	384	134
1893	996715	978	105	303508	517	170	304519	370	121
1894	1060000	1660	173	311597	421	135	211168	349	112
1895	1100000	1454	146	319000	173	54	330172	170	51
1896	1125000	1319	127	326800	154	44	342300	107	31
1897	1162749	998	94	332773	150	45	368485	115	31
1898	1197100	745	68	341651	172	50	379268	85	22
1899	1231548	744	65	349628	110	31	388587	88	23
1900	1169000	862	73	368006	46	12	391927	58	15
1901	1205000	732	60	378541	101	27	404773	57	14
1902	1243000	762	61	388930	117	30	416919	63	15
1903	1281000	803	62	399126	113	28	518405	76	16
1904	1321000	706	53	409322	114	28	533017	108	20
1905	1362000	594	43	425000	95	22	503000	74	14

* Free distribution of antitoxin from the time of Brooklyn's consolidation with New York, 1896.

TABLE XVII.

	Frankfort.			*Glasgow.*			*Hamburg.*		
Year	Population	Deaths from diphtheria and croup	Deaths per 100,000	Population	Deaths from diphtheria and croup	Deaths per 100,000	Population	Deaths from diphtheria and croup	Deaths per 100,000
1878	122292	79	65	—	—	—	371843	244	65
1879	126000	52	41	—	—	—	277068	256	68
1880	136831	33	24	—	—	—	410127	286	70
1881	139710	46	33	511415 census	—	—	416295	282	67
1882	140000	53	38	517000	—	—	416819	329	79
1883	143300	41	29	522000	—	—	435964	336	77
1884	144600	73	50	528000	—	—	449414	443	98
1885	153038	86	56	533000	—	—	462278	509	110
1886	156577	123	80	538000 approximate	211	39·1	474370	580	122
1887	160116	230	143	543000	291	53·6	486462	567	117
1888	163655	162	99	548000	274	50·0	498554	466	94
1889	167194	228	130	553000	298	54·0	510646	475	93
1890	170733	285	168	558000	203	36·3	522738	352	67
1891	183804	285	156	565710 census	194	34·4	571689	216	38
1892	188050	278	149	669059	249	37·2	594273	226	38
1893	193440	268	134	677883	310	45·8	612923	362	59
1894	198238	208	105	686820	306	45·1	636194	385	60
1895	226000	67	30	695876	185	26·6	618945	133	21
1896	233000	46	20	705052	137	19·4	631660	95	15
1897	238684	29	12	714919	145	20·3	642131	113	17
1898	244808	23	9	724349	132	18·2	652606	100	15
1899	250932	46	18	733903	123	16·8	663073	109	16
1900	264645	33	12	743963	149	20·0	673794	115	17
1901	294052	30	10	764467	123	16·1	715093	116	16
1902	302731	44	14	775601	127	16·4	731130	183	25
1903	311410	38	12	787897	118	15·0	747167	160	21
1904	320089	49	15	798357	104	13·0	763204	130	17
1905	330000	30	9	810000	48	6·0	790000	84	10

TABLE XVIII.

	Königsberg.			*Liverpool.*			*London.*		
Year	Population	Deaths from diphtheria and croup	Deaths per 100,000	Population	Deaths from diphtheria and croup	Deaths per 100,000	Population	Deaths from diphtheria and croup	Deaths per 100,000
1875	—	—	—	—	—	—	3482306	1312	37·7
1876	—	—	—	—	—	—	3538246	987	27·9
1877	—	—	—	—	—	—	3595085	855	23·8
1878	128096	263	205	534000	299	56	3652837	1150	31·5
1879	132028	197	150	541000	201	37	3711517	1169	31·5
1880	140932	179	128	548000	238	43	3771390	1108	29·4
1881	141560	262	178	552508	193	35	3824980	1346	35·2
1882	146678	352	241	549000	206	38	3862956	1707	44·2
1883	150350	287	191	546000	223	41	3901309	1771	45·4
1884	154000	249	161	543000	252	46	3940042	1698	43·1
1885	150297	286	190	540000	346	64	3979160	1580	39·7
1886	152345	154	101	537000	267	50	4018666	1374	34·2
1887	154393	179	120	534000	209	39	4058565	1481	36·5
1888	156441	122	79	531000	174	33	4098860	1800	43·9
1889	158489	211	133	527000	186	35	4139555	1784	43·1
1890	159537	194	122	523000	211	41	4180654	1885	45·1
1891	162739	92	50	517980	119	23	4223720	1858	44·0
1892	164996	173	105	518000	109	21	4269634	2241	52·5
1893	167099	179	107	518000	95	17	4212623	3454	80·1
1894	169202	162	95	*638291	127	24	4351501	2873	66·0
1895	171497	86	50	641000	170	27	4387248	2488	56·7
1896	174094	91	52	663633	202	32	4419411	2804	62·0
1897	176595	74	42	669243	149	22	4447907	2345	52·0
1898	178821	17	9	669243	164	23	4476640	1823	40·8
1899	181047	16	9	774912	242	36	4493617	2028	45·0
1900	185987	37	20	680628	183	30	4510711	1620	36·0
1901	191429	32	16	686332	209	30	4544983	1385	35·0
1902	194766	524	115	*710337	241	34	4579110	1199	26·0
1903	198103	121	61	716810	177	24	4613812	778	17·0
1904	201440	86	42	723430	214	28	4648950	754	16·0
1905	197000	44	22	723000	214	28	4700000 (app.)	573	12·2

* City boundaries extended.

TABLE XIX.

	Munich.			*Paris.*		
Year	Population	Deaths from diphtheria and croup	Deaths per 100,000	Population	Deaths from diphtheria	Deaths per 100,000
1878	222000	298	130	—	—	—
1879	225000	293	130	—	—	—
1880	229854	367	160	2225000	2048	92
1881	233000	394	169	2239938	2211	90
1882	238000	263	110	2244000	2244	100
1883	240000	272	113	2248000	1781	79
1884	242000	182	75	2251000	1928	85
1885	259318	176	68	2356000	1655	73
1886	265710	223	86	2260945 census	1512	67
1887	272102	204	75	2280000	1585	70
1888	728494	264	36	2300000	1729	75
1889	284886	401	141	2340000	1706	73
1890	291278	303	104	2380000	1668	70
1891	352718	340	96	2424705	1531	63
1892	372418	308	83	2444000	1403	59
1893	386202	258	66	2460000	1266	51
1894	399986	284	73	2480000	1009	40
1895	402459	208	51	2500000	421	17
1896	412000	185	44	2511629 census	444	18
1897	425087	182	42	2540000	298	12
1898	436430	181	41	2570000	259	10
1899	452248	104	23	2600000	236	13
1900	499932	110	22	2630000	294	11
1901	510044	80	20	2660494 census	628	24
1902	527379	68	12	2690000	529	20
1903	544714	74	13	2730000	396	15
1904	562049	89	15	2780000	250	9
1905	579000	84	14	2660000	163	6

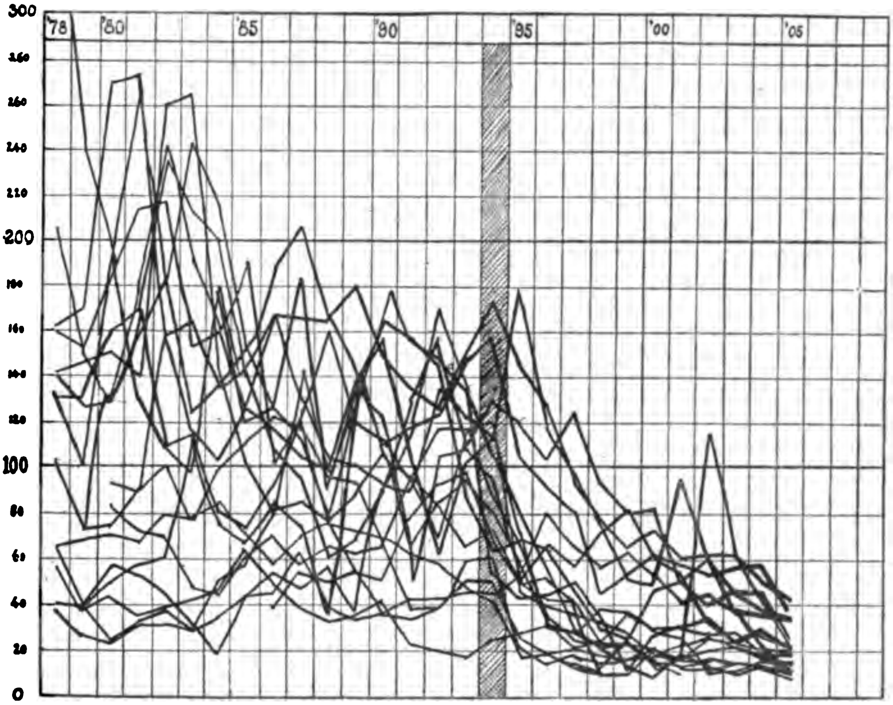
TABLE XX.

	Pittsburg[1].							*Vienna.*		
Year	Population		Deaths from diphtheria		Deaths from croup	Deaths from diphtheria and croup	Deaths per 100,000	Population	Deaths from diphtheria and croup	Deaths per 100,000
1878	145000		483		12	495	340	—	—	—
1879	150000		354		5	359	240	—	—	—
1880	156381		311		missing	311*	200	721016	597	83
1881	165000		210		,,	210*	130	741208	539	73
1882	170000		185		,,	185*	109	749919	522	70
1883	175000		170		,,	170*	98	750762	360	48
1884	185000		321		,,	321*	180	759849	342	45
1885	202559		243		,,	242*	120	769889	464	60
1886	205000		249		11	260	127	780066	546	70
1887	210000		281		103	384	183	790381	455	58
1888	220000		126	Diph. & croup	72	199	90	800836	521	65
1889	230000			333		333	138	811434	513	63
1890	238617			394		394	164	822176	536	65
1891	247000			388		388	157	1378530	1311	95
1892	255000			372		372	145	1406933	1580	112
1893	264000			233		233	88	1435931	1615	112
1894	272000			175		175	64	1465637	1679	114
1895	275000			187		187	68	1495764	710	47
1896	282500			183		183	64	1526623	621	40
1897	287500			135		135	46	1551129	575	37
1898	298772			89		89	30	1590295	520	32
1899	306115			89		89	29	1623134	463	29
1900	321616	census		147		147	46	1656662	306	18
1901	330000			165		165	50	1691996	387	22
1902	340000			170		170	50	1726604	438	25
1903	350000	estimated		215		215	60	1744177	426	24
1904	360000			166		166	46	1797992	386	21
1905	370000			98		98	26	1877000	254	19

[1] Serum laboratory opened in the autumn of 1895. Antitoxin distribution, free and unrestricted, began about Sept. 1896. Its use became general during the later part of 1896.

* The figures for croup are missing in these years; the above figures are therefore somewhat lower than they should be.

These figures are much more striking if arranged in the form of a curve as follows:



Deaths from Diphtheria and Croup per 100,000 in the 19 large cities quoted, 1878—1905.

We see from these figures that although there were marked fluctuations in the absolute mortality per 100,000 in the pre-antitoxin years, in no period did all of the cities show a decrease. Thus in 1884 about half of the cities show a decline and the other half an increase in mortality; the same is true for 1888. Not until we come to the critical year, 1894, do we find almost all the cities showing a like behaviour, a drop in the mortality per 100,000. This drop, moreover, has continued until the present time.

Two somewhat sharp rises during the antitoxin years, one in 1900 and the other in 1902, attract our attention. One of these took place in Boston, where a moderate epidemic occurred. In the course of the next two years, however, the mortality had again reached a low point. The other occurred in 1902 in Königsberg and is particularly instructive.

In 1901 there had been 16 diphtheria deaths per 100,000 population, in 1902 there were 115, and in the following three years 61, 42, 22, respectively. Here we see that the disease, assuming epidemic proportions in 1902, was not entirely checked until after three years had elapsed. In a discussion which took place on this subject at Königsberg in 1903, all the speakers agreed that the reason for the spread of the disease was the failure to use immunising injections. The city authorities did not supply the serum free and the patients usually objected to the expense. In consequence of this very few immunising injections were carried out. (See *Deutsche med. Wochenschr.*, *Vereinsbeilage*, No. 10, March 5, 1903.)

The curves shown in the preceding chart can be combined. This has been done by adding together the diphtheria deaths in the 19 cities each year and comparing the number so obtained with the total population of these cities in the same year. The results are seen in Table XXI (p. 611) and in the following curve.

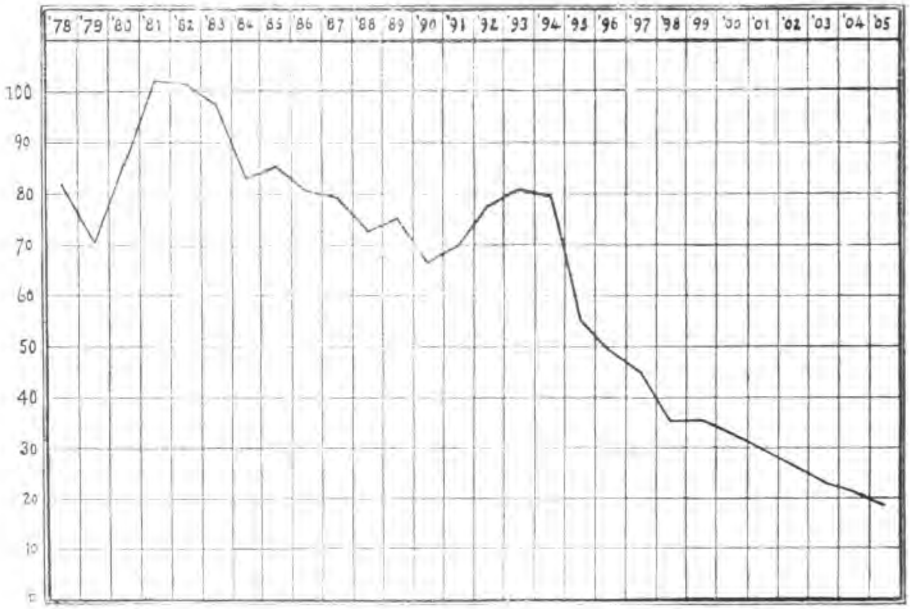


Chart showing the combined curve for the 19 cities of which diphtheria mortality returns have been quoted.

TABLE XXI. *Combined statistics of Deaths and Death Rates from Diphtheria and Croup in New York, Brooklyn, Boston, Pittsburg, Baltimore, Philadelphia, Berlin, Cologne, Breslau, Dresden, Hamburg, Königsberg, Munich, Vienna, London, Glasgow, Liverpool, Paris, Frankfurt.*

Year	Population	Deaths from diphtheria and croup	Deaths per 100,000
1878	10,000,598	8185	81·8
1879	10,188,268	7205	70·7
1880	13,401,394	11526	86·0
1881	13,642,366	13897	101·9
1882	13,857,726	14075	101·6
1883	14,049,727	13721	97·6
1884	14,353,102	11930	83·2
1885	14,544,489	12399	85·2
1886	15,617,867	12385	80·8
1887	16,217,823	12721	79·5
1888	16,300,948	11798	72·7
1889	16,526,135	12247	75·1
1890	16,526,135	11059	66·9
1891	17,689,146	12389	70·0
1892	18,330,737	14200	77·5
1893	18,467,970	15726	80·4
1894	19,033,902	15125	79·9
1895	19,143,188	10657	55·6
1896	19,489,682	9651	49·5
1897	19,800,629	8942	45·2
1898	20,037,918	7170	35·7
1899	20,358,857	7256	35·6
1900	20,764,614	6791	32·7
1901	20,874,572	6104	29·2
1902	21,552,398	5630	26·1
1903	21,865,299	5117	23·4
1904	22,532,848	4917	21·8
1905	22,790,000	4323	19·0

The statistics for Vienna do not begin until 1880; those for Glasgow until 1886. The figures for Paris are those of diphtheria only.

The extent to which antitoxin is used.

In order to ascertain the extent to which antitoxin is used at the present time we studied the question in New York City, by investigating all the cases of diphtheria occurring during a period of about three weeks in November and December 1905.

The total number of cases reported to the Health Department during that time was 385. All but sixty of these reports stated that antitoxin had been administered. In nine of these 60 cases, however, antitoxin was subsequently administered, leaving 51 cases which did not receive antitoxin, and of the latter six were not confirmed by bacteriological examination, leaving only 45 cases of true diphtheria in which no antitoxin was given.

Of these cases three died. Two were severe cases, without any physician in attendance, and one a laryngeal case, which died late in the disease, the day after a physician was first summoned.

With the exception of the fatal cases just mentioned, and one case reported as "moderately severe," all of the "non-antitoxin" cases were mild, and this fact in almost all the instances determined the decision of the physician in not administering antitoxin.

This investigation shows, therefore, that about one-eighth of the patients who are reported to be probably suffering from diphtheria do not receive antitoxin, and that this remedy is used in almost all the severe cases attended by physicians.

The preceding tables cannot be compared with each other.

While the preceding tables serve as an accurate index of the value of the serum treatment, they cannot be used for comparing the mortalities in different cities without correction for age distribution. Since diphtheria is especially a disease of childhood, it is obvious that the relative proportion of children in a community greatly affects the death rate from the disease. Since our tables were not prepared for the purpose of making such comparisons, we shall content ourselves with merely calling attention to this factor. The following table, kindly compiled for us by Dr Roger S. Tracy, gives some idea of the influence exerted by age distribution. In compiling this table he has taken the total number of deaths from diphtheria and croup amongst children under 15 years of age in New York in 1902 as the basis of the calculation. This number was 1965 out of a total of 2015 deaths. The table shows that in these differently constituted populations the crude or general death rate may vary more than 50 %, while the actual mortality remains the same.

TABLE XXII.

City	Date of census	Population by census	Population under 15 by census	Percentage under 15	Estimated population 1902	Estimated under 15	New York's diph. death-rate under 15	Calculated diph. deaths under 15 according to New York rate for 1902	Corresponding general death-rate from diph. 1902
Berlin	1900	1888577	486057	25·74	1909731	491590	1·75	860	·45
London	1901	4536541	1357874	29·93	4579110	1370600	1·75	230	·52
Munich	1895	407307	98678	24·47	509000	124570	1·75	218	·43
Paris	1901	2657335	501512	18·87	2695065	508750	1·75	890	·33
Vienna	1900	1674987	455790	27·22	1701989	471200	1·75	823	·48
New York	1900	3437202	1053298	30·64	3665735	1123400	1·75	1965	·53

CHAPTER XIX.

SERUM SICKNESS.

The clinical effects of diphtheria antitoxin. Short summary of the chronic toxic effects in man of injections of antitoxic or normal horse serum. The phenomena of serum sickness considered in detail. The stage of incubation. The fever. The rashes. The swelling of the lymph nodes. The blood changes in untreated diphtheria, in cases injected with antitoxic serum, and in cases developing serum sickness. Joint pains. Oedema and albuminuria. The condition of the mucous membranes. Rarer manifestations. Repeated injections of serum. Hypersensibility caused by serum injections. Late toxic effects produced in animals by the injection of heterologous serum. Differential diagnosis. The treatment of and prophylactic measures against serum sickness. The results obtained by the use of antitoxic globulin solution. Summary and conclusions upon the value of "refined" antitoxin. Calcium salts as a preventative of serum sickness. Theories as to the cause of serum sickness. Sudden death after an injection of serum. Duration of immunity. Repeated injections of antitoxin.

THE CLINICAL EFFECTS OF DIPHTHERIA ANTITOXIN.

THE clinical effects of diphtheria antitoxin manifest themselves both on the general symptoms due to the toxaemia of the disease and on the pseudo-membrane.

In cases which are treated early the general condition of the patient becomes noticeably bettered, the constitutional symptoms of toxaemia disappear, the colour and appearance of the patient improve, the appetite returns and mental depression disappears. When, owing to the omission of early serum treatment, toxaemia has become well marked and cellular poisoning is manifest, the above effects are much less noticeable, or take place more gradually.

If careful observations are made a rise in temperature of one or two degrees coming on four or five hours after injection is noticed in a good proportion of cases. This phenomenon is transient, lasting only a few hours, and generally escapes observation when the temperature is recorded twice daily in the routine manner.

Together with the temporary rise of temperature the pulse is also accelerated, usually out of proportion to the temperature, and the acceleration is apt to persist after the fall of the latter for a variable time. In a certain number of cases not only is there a markedly quickened pulse, but a weak, irregular heart action follows shortly after the injection. The temporary elevation of temperature is now known to be due, not to the effect of antitoxin as such, but to the proteids in its vehicle, the horse serum. Experiments have shown that the same rise of temperature occurs with non-immunised horse serum; and it has also been found that with antitoxic serum of greater concentration this effect on pulse and temperature is somewhat less marked than when an equal number of units of less concentrated serum is used.

In favourable cases, and especially in uncomplicated diphtheria, the temperature is not as a rule high and after an injection falls rapidly, becoming practically normal in three or four days. This, however, often happens even without the injection of antitoxin. In cases of mixed infection, and also in laryngeal cases with some bronchial involvement, the drop in temperature is much less sudden and takes place by lysis. When the temperature does not fall in the regular way, it is usually an indication of an otitis, a pneumonia, or other complication. Upon broncho-pneumonia, even when partly due to diphtheria bacilli, antitoxin has but little effect.

The local effects of the absorbed antitoxic serum on the recently produced pseudo-membrane are marked. In early cases of diphtheria of the pharynx or tonsils the pseudo-membrane ceases to extend six to twelve hours after a sufficiently large injection, and becomes blanched, or the dirty colour, if present, becomes less marked. The membrane appears to swell, and becomes whiter and seemingly thicker. Later the membrane becomes loosened at the edges, rolls up and soon, either spontaneously or following irrigation, detaches itself, either *en masse* or in small pieces. The process takes place somewhat more rapidly on the tonsils than elsewhere in the pharynx. The time required for the complete restoration of the mucous membrane in early treated and favourably progressing cases, when epithelial necrosis has not been extensive, varies from twenty-four hours to three or four days. Occasionally the pseudo-membrane, after disappearing, is partially reproduced, in which case a second dose of serum causes the secondary membrane to undergo the changes mentioned above. In those cases of diphtheria in which the pyogenic streptococci or other bacteria play an important part the effects of the antitoxin are not so well marked, and the exudate or pseudo-membrane persists for some time. When the lesions have lasted for some days and the superficial epithelium has become necrotic, there remains for several days after the separation of the membrane an ulceration which is coated by a thin exudate. A week or ten days is required in these cases for complete local recovery.

In nasal diphtheria, the effects are similar, the irrigation, if practised, bringing away small or large pieces of detached membrane, or sometimes a complete cast of the cavity. The nasal discharge and swelling are usually soon ameliorated and mouth-breathing ceases. In laryngeal diphtheria, when given early, the serum checks the process so that in many of the cases operation does not become necessary. If intubation or tracheotomy has to be practised, the membrane is soon loosened in favourable cases and is coughed up. The shorter average time for which the tube is required in intubation and the cannula in tracheotomy has been noted by many. It is a notable fact that the intubation tube is more often coughed out in cases treated with antitoxin than in those treated by any other method.

The gland lesions are usually favourably influenced by antitoxin treatment, especially in cases of diphtheria in which progressive mixed infection is not marked, although a moderate swelling of the cervical glands is apt to persist for some time after the disappearance of the local symptoms.

The heart is only indirectly helped by the serum, and cardiac failure remains the chief cause of death in uncomplicated diphtheria. Children and adults still die of heart weakness in spite of treatment, but this occurs in proportion to the amount of poisoning which had taken place before sufficient antitoxin was given and the degree to which the heart had already been poisoned. If the heart muscle is already diseased the antitoxin cannot restore it. The same is true of the kidney. The effect of the horse serum on the kidneys is considered under serum sickness.

THE CHRONIC TOXIC EFFECTS IN MAN OF INJECTIONS OF ANTITOXIC OR NORMAL HORSE SERUM. "SERUM SICKNESS."

Ordinarily, with the exception of a little local irritation, the injection of antitoxin is not followed by any immediate local changes.

Abscesses at the site of inoculation only arise if insufficient care has been used to guard against septic infection, or in cases suffering from septicaemia where the presence of the serum may cause a localisation of the pus organisms. If the hands, syringe, vessels, etc. are clean abscesses very rarely develop, provided the serum is sterile, and even if it is not, infection frequently does not follow.

It was early noted, however, that injections of antitoxin were sometimes followed after a few days by a peculiar train of symptoms, principally rashes, pains in the joints and fever. One of the first to publish his observations was Lublinski (1894). His patient was an eight year old girl who received three injections of 10 c.c. each on the second and third days of the disease. On the fifth day of the disease there was reddening of the site of injection. Nine days after the last injection high fever and painful swelling of the joints developed, accompanied by an extensive macular exanthem of the multiform type. This condition lasted about four days. The child made a good recovery.

Shortly afterwards Scholz (1894) reported four examples of this condition, and since that time a large number of authors have described serum rashes.

Even at that early date both Heubner and Bokay expressed the view that the phenomena were due to other constituents of the serum than the antitoxin, and this was subsequently confirmed experimentally by Johannessen. By injecting normal persons with normal horse serum he produced the same phenomena. Of 22 normal persons so injected eight suffered from fever and 12 from exanthems.

Hartung (1896) collected statistics on the frequency of serum rashes and found that they occurred in from 8 to 11% of the cases. He considered that the frequency was dependent more on the individuality of the horse furnishing the serum than on the quantity of serum injected. Since that time this subject has received much attention and is of sufficient importance to require detailed consideration.

In the following account we have made free use of the excellent study on serum sickness published by v. Pirquet and Schick (1905).

Apart from a little local irritation no immediate changes are usually noticed even after large injections; and during the next few days the site of injection shows absolutely no reaction. There is nothing to denote that a foreign substance has entered the body, for the patient's general condition is not disturbed. Then in about 20% of the cases, usually between the 8th and 12th day, symptoms of disturbance suddenly manifest themselves.

Now and then *prodromal signs* precede the general outbreak of the symptoms by a few days. The skin is sensitive to even slight irritation and shows indistinct reddening; this is especially the case at the site of injection and in its vicinity, which is reddened and itchy. The most constant of the prodromal symptoms is a slight swelling of the nearest lymph nodes.

As a rule the first indication of the disease itself is the development of an eruption, generally urticarial in character, which usually spreads from the site of injection over the rest of the body. In most instances it appears simultaneously in symmetrical places. Some of the wheals are pale, some surrounded by a livid red zone. The latter is especially the case if the patient scratches a good deal. If the lesions are close together, areas in which the eruption is confluent are often seen. In such cases the entire area seems to be oedematous, and if occurring on the face often gives rise to marked disfigurement. Owing to the intense itching the patients are restless and out of sorts.

All of the eruptions are fleeting. A particular wheal only lasts a few hours, and the entire urticarial rash rarely lasts longer than from two to three days. There is no regularity in the course of the eruption such as is seen, for instance, in smallpox.

In the meantime the temperature rises to some extent, showing that the rest of the body is taking part in the reaction. The intellect and special senses, however, are unaffected. With the rise of temperature the pulse rate is quickened, but the quality of the pulse remains good.

In addition, however, to the cutaneous symptoms to which the name "serum rash" has been given, there are others. In the cases which react the lymph nodes draining the site of inoculation gradually enlarge from the period of incubation. With the onset of the fever and rash this swelling quickly increases in intensity, and other lymph nodes may become affected later. The swelling, which is painful and tender to the touch, subsides when the other serum phenomena are disappearing.

Especial attention must be called to the state of the renal functions and to the development of slight *oedema*. The latter is quite regularly observed, and is not only visible, but can be demonstrated by the increase in body weight according to the method of v. Pirquet. With the development of the disease the curve of body weight increases despite the fever and the decreased absorption of food. The maximum of the curve usually coincides with the height of the symptoms. After the oedema has reached a certain intensity, it is appreciable to the eye and to the palpating finger. The oedematous areas correspond in their localisation to those which follow nephritis.

In spite even of marked oedema there are usually no urinary changes which indicate disease of the kidneys. Only in a minority of cases does an excretion of albumin in the urine occur, and even in these cases the amount always remains small. v. Pirquet and Schick for example never found more than ·025 %. A slight sediment only occurs, which contains merely a few hyaline casts and some red blood corpuscles. As the body weight increases at the time of the oedema, there is a reduction in the amount of urine excreted, but there is never anuria.

Compared with the constancy of the above-named symptoms the others are much less frequent. Of these the joint pains, which occur in about 1 % of the cases, have always been regarded as one of the most prominent. The various kinds of rashes, the rare mucous membrane symptoms and the blood changes will be discussed later (p. 621).

The *general condition* of the patient is usually not much affected, especially when the disease lasts but a short time; in fact the patient's feelings are often in marked contrast to the high fever. Rare cases, however, occur in which after injections of large quantities of serum the disease lasts from four to five weeks. These patients often suffer from severe prostration and loss of weight. As soon as the disease ceases, however, and convalescence begins, the lost weight is rapidly regained and the disease disappears without leaving any sequela. No after effects are observed, and not one of the individual symptoms can lead to permanent injury.

v. Pirquet and Schick have never seen a fatal ending which could be ascribed to the action of the serum. At the most they concede that serum sickness developing in a patient who is extremely ill may so lessen his resistance that he succumbs to the combined attack. Several fatal cases (p. 636) have, however, been recorded in which death has apparently been due to the injection of serum, but in these the fatal ending has occurred within a few minutes of the injection.

It is very difficult to give a good general picture of serum sickness, because the variations in the symptoms are dependent not only on the individual disposition of the patient but also on the nature and amount of the serum injected. The cases of serum sickness which are the most frequent, and therefore practically of the most importance, are rudimentary ones, in which only one or another symptom is well marked. The most frequent are those showing fever and exanthem.

THE PHENOMENA OF SERUM SICKNESS CONSIDERED IN DETAIL.

The Stage of Incubation.

In serum sickness, as in a large number of diseases, the symptoms do not, as a rule, come on immediately after the subcutaneous or intravenous injection of the serum. In other words, between these two events there is a definite interval—the stage of incubation—during which there are no symptoms.

Naturally we disregard the temporary slight swelling, tenderness and redness at the site of injection and the immediate vicinity. These are merely the results of the injury incidental to the injection.

The incubation period is not due to the slow absorption of the foreign serum, for it is present when the serum is given intravenously. Hartung observed that in more than half of his cases the serum symptoms came on ten days after the injection. v. Pirquet and Schick (1905) studied 90 cases of serum sickness following a first injection and found the average duration of the incubation period to be from 8 to 12 days. In patients injected a second time the symptoms occur after a shortened period of incubation. We have several times observed that with the sera of certain horses early rashes are very frequent, often occurring on the second day.

The Fever.

Corresponding with the varying intensity of all the symptoms the course of the fever in serum sickness is irregular. In spite of its irregularity, v. Pirquet and Schick (1905) believe that they have established the constancy of the following points, especially in cases in which large amounts of serum have been administered.

1. Fever is one of the most frequent symptoms, occurring more often than the rashes.
2. It lasts until the end of the clinical symptoms of the disease.
3. In the more severe cases, the type is generally that of a remittent fever, the daily fluctuations amounting to 1—3° C. Less frequently morning temperatures are also high. Toward the end of the disease, when the morning temperature is frequently normal or subnormal, the curve of the fever often becomes intermittent.

4. There is a certain relation between the course of the fever and the form of the rash. The highest fever is seen with the morbilliform (maculo-papular), the lowest with the erythematous rashes. The urticarias and the scarlatiniform rashes occupy an intermediate position. [In our own experience the scarlatiniform rashes have been accompanied by as high fevers as the morbilliform.]

5. The height of the temperature does not, in general, permit of any conclusions as to severity or duration of the disease. [In our experience there has been considerable correspondence.]

6. The duration and intensity of the fever are dependent partly on the amount of serum injected and special horse from which it was obtained, and partly on the individual disposition of the patient.

In regard to sections 1 and 2, it should be added that at the time the serum reaction is complete and temperature elevated the rashes may still be absent. In order to avoid misunderstanding we must add, however, that in spite of our emphasis on the presence of fever, there are many cases in which the fever is entirely absent. Further we must point out that the fever must not be regarded as the result of the rash. Fever and rash are in fact coordinated effects of one cause.

High fever of short duration is frequently seen to follow the injection of small amounts of serum.

In discussing section 6 some help may be obtained from statistics. The statistics for the early antitoxin days, when the injections amounted to 10—30 c.c. of serum, show that 22 % of the cases developed symptoms of serum sickness. In recent years, with injections of from 5—15 c.c. the proportion of cases suffering from serum sickness has fallen to 6·5 %. Many observers, however, still record a high proportion. Even at the present time, however, when large injections of serum amounting to 100 to 200 c.c. are practised (Moser's scarlet fever serum), serum symptoms are met with in 85 % of the cases.

That there is a peculiarity in predisposition to the disease is shown by the fact that brothers and sisters may often exhibit the same anomalous behaviour in response to injections, as, for example, an abnormally short incubation period.

The Rashes.

The rashes are extremely varied in character, and are divided by Hartung (1896) into the following four groups:

(1) Urticarial.

(2) Scarlatina-like rashes.
  (*a*) Diffuse erythematous.
  (*b*) Finely sprinkled (true scarlatiniform).
(3) Morbilliform.
(4) Polymorphous, including exudative forms.

Almost every observer, however, must have encountered cases which could not be classified under these many varieties. v. Pirquet and Schick (1905), therefore, believe that it is unwise to attempt a separation into these groups, since many intermediate forms are observed. These authors believe that the exciting cause of the rash has less to do with the form of the eruption than the condition of the patient. An individual may even have several rashes of different forms following an injection of serum. In the majority of cases the primary rash belongs to the urticarial class. In some cases there are fresh eruptions on new areas every day until the entire skin surface has been traversed. If, after this form of eruption has come to an end, a new type develops in its place it is the rule that the latter contains no urticarial spots. A similar rule is observed when a second and third different kind of rash develops.

Our own experience leads us to believe that the individuality of the serum often plays a prominent part in the type of rash which is produced. We have observed that after the injection of certain sera the majority of rashes are morbilliform or scarlatiniform. Nevertheless, we wish to clearly state that we do not claim that the different kinds of rashes are produced by different constituents of the serum. If this was the case we should have to assume that each of these substances possessed a distinct period of incubation, since the morbilliform rashes almost invariably follow the urticarial and the reverse order is rarely or never observed. If general rashes develop, they are almost always of the urticarial type.

Owing to the fleeting character of the rashes it is difficult to watch their course over the body. In the case of the urticaria the rash frequently first appears as a reddening of the skin accompanied by itching, and later a wheal develops in the centre of the area. A rash generally takes the following course:

(1) The rash usually first appears at the site of inoculation, and not infrequently this local rash is the only symptom, so far as the skin is concerned.

(2) The rashes on the rest of the body usually develop symmetrically.

(3) The urticarial rashes are accompanied by marked itching.

(4) The morbilliform and exudative rashes occur most frequently on the extensor surfaces of the extremities.

For the sake of completeness we shall merely state that two other types of rash are occasionally met with. The first of these is the cachectic rash, which is seen in debilitated individuals. The rash appears pale and yellowish, and, especially in the urticarial forms, is but slightly raised from the surface with only a trace of the usual red surrounding zone. The other form is the haemorrhagic rash, which is only very rarely met with.

The points to be considered in the differential diagnosis between the serum rashes and some of the exanthemata are discussed later (p. 629).

(1) *Urticaria.*

Urticaria usually appears first at the site of injection but soon spreads over the trunk and limbs. Sometimes only a single wheal may occur but usually the condition becomes generalised and successive crops may appear every few hours. There may be considerable oedema in the vicinity of the wheals. The rash as a rule does not persist long. The irritation produced by the itching may be very troublesome causing insomnia, especially in neurotic patients.

(2) *Erythema.*

Erythema usually occurs as a secondary rash following the urticaria, its onset occurring from about the twelfth to the twenty-ninth day. The erythema is at first amorphous but tends to become circinnate and form gyrate patterns. The rash usually appears first on the knees and elbows or on the inner side of the thighs, and may subsequently spread to the hands and face. Urticaria and erythema may occur simultaneously.

(3) *Scarlatiniform.*

A scarlatiniform rash is comparatively rare and is frequently limited to the site of injection. Leiner (1902) states that it is contagious and believes it to be a true scarlatina. Most observers, however, attribute a rash of this type to the antitoxin injection.

(4) *Morbilliform.*

When the circinnate erythema affects the face the resemblance to measles is striking. This is especially the case when it is accompanied by congestion of the conjunctiva, lachrymation and swelling of the face, a not infrequent occurrence.

Relative Frequency of the different Types of Rash.

Great divergence of opinion exists on this subject. Thus Poix (1896) states that Lebreton and Magdelaine observed 54 urticarias, 12 scarlatiniform eruptions, two rubeoliform eruptions and two polymorphic erythemas. Sevestre and Meslay recorded 22 urticarias, four scarlatiniform and one erythema multiforme. By classifying the results of the observations of 50 authors Dubreuilh obtained records of 156 urticarias, 46 scarlatiniform erythemata, 11 rubeoliform erythemata, and 31 polymorphic erythemata.

Stanley (1902) found that erythema was the commonest form in the Metropolitan Asylums Board Hospitals, London, whereas Rolleston (1905) working in the same hospitals at a later period, and using antitoxin from the same source, found that "the character of the rashes had changed," urticaria being then most frequent. Stanley out of 112 cases found:

Erythema	58
Urticaria	30
Scarlatiniform	6
Morbilliform	under 3 %

Rolleston out of 289 rashes which appeared from the first to the 10th day inclusive found:

Scarlatiniform	11
Circinnate erythema	6
Urticaria	272

and out of 94 later rashes:

Circinnate erythema	84
Urticaria	10

The Swelling of the Lymph Nodes.

Swelling of the regional lymph nodes is a more or less constant symptom of serum sickness. Injections of serum under the skin of the abdominal wall usually cause swellings of the inguinal glands of the same side. If the reaction is more intense the axillary glands may also be affected. The general course of this glandular enlargement in cases of serum sickness is as follows:

Beginning about the 7th or 8th day after the injection a gradual increase in the size of the lymph nodes nearest the site of injection occurs. Glands which were at first just palpable become as large as peas, and stand out distinctly when the hip joint is extended.

With the appearance of the general serum symptoms there is a rapid increase in the size of these glands; they frequently become as large as a date stone, or more, and stand out as one mass. At this time they are usually painful and tender to the touch. As a rule the swelling increases still more during the first few days and often extends to other lymph glands. Though the swelling remains until near the end of the disease, the tenderness disappears sooner. When the glands begin to decrease in size, it may be concluded that the disease is about to end. v. Pirquet and Schick (1905), therefore, lay stress on this as a prognostic point.

The Blood Changes in Untreated Diphtheria, in cases injected with Antitoxic Serum, and in cases developing Serum Sickness.

The blood changes in diphtheria (without the development of serum sickness) have been carefully studied by Ewing (1901), who found that the disease caused a slight anaemia with very few morphological changes in the cells. The leucocytes usually show a considerable increase in numbers, 25,000—30,000 per c.mm. being frequently present in severe cases. The majority of fatal cases at some period show 25,000—50,000 leucocytes per c.mm. Mild attacks, especially in adults, may not produce any increase. In cases treated with diphtheria antitoxin the blood changes are less marked. Within half an hour after the injection of antitoxin, the leucocytes, especially the polynuclear forms, if previously abundant, show a marked diminution, and in most cases, although the leucocytosis returns after 24—48 hours, it seldom reaches its previous height. Billings (1896) found that the injection of antitoxin into healthy individuals produced a slight reduction in the number of blood corpuscles in about one half of the cases. The haemoglobin was correspondingly affected, but the leucocytes were apparently unaffected by the injections. Field in our laboratory obtained practically identical results in investigations on two dozen normal children injected with either ordinary or "refined" antitoxin (Gibson's process).

v. Pirquet and Schick (1905) describe a marked leucopenia developing with the onset of symptoms in many cases which suffer from the serum disease, after large or moderate doses. The decrease, which is particularly at the expense of the polynuclear cells, resembles closely the leucopenia observed in measles, scarlatina and vaccinia.

Joint pains.

Joint pains, although not especially frequent, have attracted the attention of many observers. Hartung (1896) observed joint symptoms in 140 (6·7 %) out of 2073 cases of serum sickness.

Amongst 4358 cases cited by v. Pirquet and Schick (1905) the condition was encountered about 40 times (1 %). Rolleston (1905), quoting a report to the London Clinical Society, states that joint pains occurred in 40 (6·3 %) out of 633 cases. In all of these cases fever developed, and in 35 a rash at the same time as, or just before, the joint pains. Barbier (1904) in Paris noted joint affections in six out of 763 cases. The symptoms lasted from four to eight days, and all the patients recovered between the 6th and 14th day. In our experience the joints have been involved in less than 1% of the cases which developed serum sickness.

The metacarpo-phalangeal joints are the ones most frequently affected, and next in order of frequency are the joints of the hand and the knee. Pain, which is increased on moving the joints, is a marked feature. Objectively, there is usually nothing to be found, or only slight swelling and redness, and in this respect the conditions differ from those observed in acute rheumatism. Owing to the benign course which this affection takes, pathological examinations in the human subject are not obtainable. In a calf, despite the presence of marked pains, no pathological changes were found, not even a trace of swelling. It is possible, since the joint pains occur at the same time as rash, that the synovial membrane of the joints is affected in the same way as the skin. The joints do not become purulent and there are no heart complications such as endo- or pericarditis. Salicylates do not influence the condition. Cold compresses seem to give the greatest relief. It must be remembered that in diphtheria patients treated both with and without antitoxin from time to time develop suppurative arthritis.

Oedema and Albuminuria.

We have already stated that the development of a slight oedema is a very frequent symptom of serum sickness. Albuminuria, on the contrary, is only rarely observed. The oedema shows itself usually in the face, and less frequently in the dependent parts of the body. We may, therefore, conclude that it is not due to passive congestion. The duration of the oedema corresponds to that of the serum sickness, and, like the decrease in the glandular enlargement, the decrease in the oedema is of prognostic value.

When albuminuria occurs it does not appear until a few days after the development of the oedema. The amount of albumin, however, is always slight, never more than about ·025% The urinary sediment contains a few hyaline casts or a few stray blood corpuscles.

The Condition of the Mucous Membranes.

The mucous membranes hardly ever take part in the serum reaction and this fact constitutes a valuable aid in the differential diagnosis from scarlet fever and measles. v. Pirquet and Schick (1905) never saw an angina which could be traced to the serum. They observed, however, not infrequently diffuse bronchitis as part of the serum symptoms, and in a few cases bloody diarrhoea.

Rarer Manifestations.

Associated with the dilated state of the vessels haemorrhage into the gut has been recorded. Gerlach (1905) attributed a case of urethral haemorrhage to leakage from the dilated vessels. Oedema of the glottis and diffuse bronchitis have also been observed.

REPEATED INJECTIONS OF SERUM.

(*a*) *Hypersensibility caused by Serum Injections.*

Arthus (1903) and v. Pirquet and Schick (1903) have drawn attention to the fact that an injection of serum into an individual who has previously received injections may give rise to more rapid and more acute symptoms than in a normal person. The latter authors have also pointed out that an increased rate of reaction or even an immediate reaction may occur. In the latter case oedema at the site of injection and frequently urticaria, pyrexia and all the other symptoms of serum disease are found within 24 hours.

They point to the analogy between this phenomenon and that observed by Richet (1904—1905) in the case of the poison congestin obtained from Actinia. Richet found that the lethal dose of this substance for rabbits at the first injection was ·009 grm. per kilo. Only ·003 grm. was required to kill an animal which had previously received an injection. The action is not due to a cumulative effect, since three to four days must elapse after the first injection before the hypersensibility develops. As a previous injection produced the opposite effect to a prophylactic action Richet called the condition "anaphylaxia" or hypersensibility.

Hamburger and Moro (1903) demonstrated precipitin in the blood serum of the human subject after the injection of horse serum, and were at first inclined to attribute the phenomena of serum disease to the formation of a precipitum within the circulation. After the demonstration of Rostoski, Michaelis, and Oppenheimer (1902) that no precipitation occurs within the living body this view was abandoned.

v. Pirquet and Schick attribute the immediate or hastened reaction and indeed the serum disease as a whole to an interaction between the antigen and the antibody. The term antibody is not to be interpreted as synonymous with precipitin. They draw far-reaching deductions as to the essential nature of immunity from these observations. During its evolution the protective mechanism of the body against disease has been chiefly directed against organisms which multiply in the body, and the quicker the reaction of the body the less time has the foreign invader to multiply. In nature the injection of poisons incapable of multiplication is so rare (*e.g.* snake-bite), that it forms an artificial type of disease. The hastened reaction is the chief advantage which the animal has derived from its survival from attacks of disease. In re-vaccination with vaccine lymph one of the striking features is the earlier occurrence of the reaction, and this, according to v. Pirquet and Schick, is the essence of the immunity by which the vaccinated individual benefits.

(*b*) *Late Toxic Effects produced on Animals by the Injection of Heterologous Serum.*

A number of workers [Arloing (1895), Murajew (1898), Salter (1898), Linossier and Lemoine (1902)] have shown that late toxic results may follow the injection of even small doses of heterologous serum, and that injurious effects are especially apt to occur if the injections are frequently repeated. Salter, for example, found that the rate of growth in young guinea-pigs was retarded by injections of horse serum. Oppenheimer (1904) got results in direct opposition to those of Linossier and Lemoine. The writer's experience is that animals repeatedly injected with normal horse serum, even after it has been heated to 56°—60° C., for half an hour, tend to become cachectic and die. This is in agreement with results recorded by v. Pirquet and Schick.

The ordinary phenomena associated with serum disease have only rarely been recorded in animals. Beclère, Chambon and Ménard (1896) observed in calves a rise of temperature accompanied by a generalised exanthema and an affection of the joints after the injection of large quantities ($\frac{1}{100}$ of the body weight) of horse serum. The rashes were of an urticarial or morbilliform type with marked infiltration of the skin. No characteristic histological changes of the skin or joints were found. Aronson (1904, quoted by v. Pirquet and Schick), has not infrequently observed a generalised papular eruption in horses following the injection of a heterologous serum.

DIFFERENTIAL DIAGNOSIS.

The similarity of the rashes of serum sickness to those of scarlet fever, measles and toxic conditions, often leads to difficulties in correct diagnosis. These difficulties are in part due to the fact that in most cases the individuals are already suffering from one disease and it is hard to tell to what extent this disease is answerable for some of the symptoms. It is very important not to make the mistake of ascribing every unexplained rise of temperature about the time that serum symptoms ought to appear to the serum.

Serum sickness has most frequently to be diagnosed from measles and scarlet fever. The following points indicate serum sickness.

1. The time of development of the rash (7—14 days after injection).
2. Rash appearing first in the vicinity of the inoculation site.
3. Regional glandular enlargement.
4. Entire absence of mucous membrane lesions.

The second point must be used with considerable caution for exceptions to it are not uncommon. Oberwinter (1903), for example, describes a number of cases in which true scarlet fever made its first appearance at the site of inoculation. One of his cases is described in detail later (p. 630).

The last point makes the diagnosis from measles quite easy. The absence of Koplik spots, coryza and conjunctivitis are sufficient to show that the patient is not suffering from measles. The diagnosis from scarlet fever presents greater difficulties. In many instances, in fact, the scarlatinal nature of the rash is not recognised until other children are attacked, or until there is a characteristic desquamation or a haemorrhagic nephritis. Owing to this fact some authors have even questioned the existence of scarlatiniform serum rashes. We believe, however, there is no doubt that such rashes do occur. Help in arriving at a diagnosis may be obtained from observing the following points.

1. Initial vomiting.
2. Presence of angina and exanthem.
3. High fever.
4. In a hospital the development of the disease in several children simultaneously or at very short intervals. This fact can be used as

a differential sign because the scarlatiniform type of serum rash is relatively rare.

The differentiation of serum rashes from food, and especially drug rashes, might often be very difficult. In practice, however, these very rarely need be considered. Owing to the time at which the serum rash develops, the patient has usually been under medical observation for a number of days, and during this time the drugs administered are known. Urticarias due to food are usually not difficult to distinguish, for gastro-intestinal disturbances generally precede urticarias of intestinal origin.

The following clinical cases illustrate some of the diagnostic difficulties.

Case 1, reported by Dr Kellogg at the Minturn Hospital, a private hospital in New York City for the treatment of diphtheria and scarlet fever, in which the scarlatina rash was supposed to be due to serum until the spread of infection showed its nature. A patient with laryngeal diphtheria had been given antitoxin and 48 hours afterward developed an erythematous rash, which soon became faintly papular, starting on the chest and spreading over the whole body. The condition of the pharynx was not characteristic of scarlet fever, but vomiting occurred. The case, though diagnosed as a scarlatiniform antitoxin eruption, was isolated. Four days later typical scarlet fever desquamation took place, and the nurse who was in charge of the case developed typical scarlet fever four days afterwards. No diphtheria bacilli were found in her throat.

Case 2, reported by Oberwinter (1903), in which two rashes at first considered to be due to serum proved eventually to be due to scarlatina.

"In November, 1901, we admitted at the same time into our Diphtheria Ward two children, Elsie F., four years old, and Gertrude F., five years old. They were cousins and had frequently played together recently. Both had sickened at the same time two days before, having probably been infected together. Both had a thick diphtheria membrane in the throat from which diphtheria bacilli were obtained in large numbers. Gertrude showed, even on admission, a marked infiltration of the cervical lymph glands. The children were immediately injected with 3000 units of antitoxin. The fever subsided rapidly and the membranes were thrown off in a few days.

On the fourth day of hospital treatment, after they had both been free from fever for two days, the temperature suddenly rose to 39° C. General condition was but little affected. At the site of injection there was seen on both children a brilliant red, finely spotted exanthem. On the trunk there was a similar, though far paler rash. The extremities and face remained free. Tongue showed a little erosion at the tip. Throat somewhat reddened, as was only to be expected after the recent throwing off of the membrane. The pulse, which in both children was still rapid, remained so (120—130) per minute.

On the following day the extremities also were affected, faces, however, remained clear. In the course of the next four days the rash faded slowly. During the entire time the general condition was but slightly affected. Only in Elsie did the tongue show any erosion. Throat only slightly reddened. In Gertrude the fever disappeared as early as the second day of the rash.

Although the long duration of the rash, as well as the accompanying fever (at least in one of the children), was very suspicious of scarlet fever, the undisturbed general health of *both* children, the development of the rash within the first three or four days, the decline of the fever (in Gertrude as early as the second day) and the almost entire absence of throat or tongue involvement, all these spoke against scarlet fever. Furthermore it would have been a curious coincidence if two typical diphtherias should be infected with scarlet fever immediately on entering the hospital, and that these should then both develop the disease at the same time and run a similar course. We therefore decided on the diagnosis of 'serum rash,' until, to our sorrow, we saw large flakes of skin scale off and, what was still more, when the patient against our wishes left the hospital at the end of 3½ weeks, she promptly infected her sister with typical scarlet fever."

v. Rittershain (1902) also observed a rash beginning at the site of inoculation, which was at first thought to be a serum rash. The patient, however, later developed typical scarlet fever.

THE TREATMENT OF AND PROPHYLACTIC MEASURES AGAINST SERUM SICKNESS.

The therapeutic treatment of serum sickness may be dismissed with the words "symptomatic treatment." Cold compresses may be applied to the swollen parts and prolonged warm compresses to the affected joints, local applications of menthol, etc. to relieve the itching, sponging for the fever, etc.

In considering the prophylactic treatment it must be remembered that the serum sickness is due to certain constituents of the serum, and that these have apparently no connection with the antitoxin, for normal horse serum produces the same effects as does antitoxic serum. As soon as this fact was duly appreciated efforts were made to purify the antitoxic serum, and, if possible, to isolate the antitoxin. For the same reason attention was directed to securing as strong a serum as possible. In the early antitoxin days a sample of serum contained only 60—70 units per c.c. By employing selected horses and strong toxins from certain strains of bacilli, especially the New York Health Department culture No. 8, a higher grade of serum was gradually obtained, so that after some years the ordinary antitoxic serum contained 200 units per c.c. in some countries and 300 units in others. With these sera much smaller injections were required to give the same dose of antitoxin. In spite, however, of the fact that the serum of many laboratories now contains as much as 400—500 units per c.c. rashes are still quite frequent.

Many workers have attempted to entirely eliminate the non-antitoxic portions of the serum, and those interested in the chemical side of these investigations are referred to the recent article by Gibson (1905). In 1900 Atkinson, working in the Research Laboratory of the New York Health Department, eliminated all but the globulin from the antitoxin, and we tried this partially refined serum in 36 cases. The results in these cases were so nearly identical with those obtained in an equal number of cases treated with the whole serum from the same horse that the preparation of this expensive antitoxic solution was abandoned. The idea that a practical separation of the antitoxin from much of the proteid non-antitoxic portion of the serum was possible was not given up. In August of 1905 we tested an antitoxic preparation which we hoped might be more successful. Dr R. B. Gibson, Chemist in the Research Laboratory, placed the ammonium sulphate precipitate from the antitoxic serum in saturated sodium chloride solution and found that the globulin soluble in this contained all the antitoxin. In this way the nucleo-proteids and the insoluble globulins, present in the Atkinson preparation, were eliminated. These soluble globulins were precipitated by acetic acid, and placed in a sac of parchment membrane and dialysed. The solution of globulins was then neutralised and sufficient sodium chloride was added.

This solution of antitoxin and a portion of the other soluble serum globulins was then tested on a number of children. From the first the results were favourable but injections caused more local pain than when ordinary antitoxic serum was used. Stricter attention to the neutralisation soon overcame this defect with the result that when serum was injected on one side and globulin solution on the other, the patient was unable to tell the one from the other. In October 1905 the antitoxic globulin solution was administered not only in the hospitals but also in private homes by the Medical Inspectors. Since December it has been gradually distributed throughout New York City and is now the only form of antitoxin supplied by the Health Department.

The Results obtained by the Use of Antitoxic Globulin Solution.

The antitoxic effects of the antitoxic globulin solution seem to be identical with those of the whole serum. This must almost necessarily be the case, for the unit of the antitoxic globulin solution is identical with the unit of antitoxin supplied by the United States Government. Our tests have shown that not only the toxin but also the poisons produced in the animal by injections with virulent bacilli are neutralised as completely by the globulin solution as by the antitoxic serum from which it is separated. Both we ourselves and the resident and attending physicians of the contagious disease hospitals noted that the rashes following the injections of the globulin solution seemed to be decidedly less severe than those which formerly followed the injection of the whole serum, and that there were very few who had any constitutional disturbances even when the rashes did develop. As the serum, supplied by different horses or even by the same horse at different times, is known to vary, and as it is therefore difficult to accurately compare different bleedings it was decided to make a test by collecting a quantity of serum from four different horses, mixing it thoroughly and then after precipitating one half to treat an equal number of patients simultaneously with the two preparations. These tests were chiefly carried out in the Willard Parker Hospital, but a few cases were also treated at the Riverside Hospital. Drs Lynah and Watson, the resident physicians in charge of these two hospitals, took great interest in these experiments and afforded valuable aid, and we wish to express our indebtedness to them. It soon became evident that the serum which we had chosen was one of such a character that eruptions and constitutional disturbances usually appeared in those who were injected. Whether this was due to some other reason or to the fact that serum from four horses had been mixed, this serum produced more after-effects than any we had used in the hospitals since 1899. These after-effects were so marked and occurred in such a large proportion of the children that we decided to stop the use of the whole serum as soon as we became aware of the fact. In those over 10 years of age rashes seldom occurred. The rashes in those inoculated with the globulin preparation were much less severe. The cases treated with both the whole serum and the antitoxic globulins were most carefully watched, and the course of the disease, as well as after-effects, noted.

After all the patients had become fully convalescent or had left the hospital the histories were finally compared. It was found that fifty children under ten years of age treated with the whole serum had lived at least nine days or long enough for the development of serum effects. The first fifty consecutive cases in children under 10 years treated with the antitoxic globulins precipitated from the same lot of serum were compared with these.

The following table gives a summary of the constitutional and local reactions observed during the treatment of 100 cases of diphtheria in young children, 50 of whom received mixed antitoxic serum from four horses and 50 a solution of the antitoxic globulins derived from the same lot of serum.

	Children who were treated with the whole serum	Children who were treated with the solution of antitoxic globulins
Marked constitutional symptoms accompanied by a severe and persistent rash in	26 °/o	0 °/o
Moderate constitutional symptoms accompanied by a well developed erythema or urticaria ...	18 °/o	4 °/o
Very slight constitutional disturbance accompanied by a more or less general rash in ...	20 °/o	8 °/o
No appreciable constitutional disturbance, but a more or less general urticaria or erythema in	4 °/o	34 °/o
No appreciable deleterious after effects whatever in	30 °/o	54 °/o

Duration of rashes.

Days:—	1	2	3	4	5	6	7	8 & over	Total
Cases treated with antitoxic globulin	5	7	5	2	3	0	1	0	23
Cases treated with whole serum ...	1	4	10	1	10	3	2	5	36

The concentration of antitoxin made possible by the elimination of the non-antitoxic substances is not only a convenience but is of distinct importance as it encourages the use of large doses.

The antitoxic globulin solution tends to become slightly cloudy when kept at moderate or high temperatures and certain substances, such as solutions of carbolic acid and trikresol, precipitate it.

Summary and Conclusions upon the Value of "Refined" Antitoxin.

The results obtained in this series of 100 cases are so definite that it seems safe to conclude that the removal of a considerable portion of the non-antitoxic proteids from the serum by the Gibson method eliminates much of the deleterious matter, so that rashes, joint pains, fever and other constitutional disturbances are less likely to occur from the injection of antitoxic globulin solution than from the injection of the antitoxic serum from which it was obtained.

The globulins accompanying the antitoxin in the Gibson preparation are capable of exciting rashes and occasionally constitutional disturbances. It is probable that methods will be devised to refine antitoxin still further and so possibly eliminate all the appreciable deleterious effects of the antitoxic serum.

Whether the globulin preparation is less liable than the serum to cause collapse in cases of peculiar susceptibility, such as those suffering from the status lymphaticus, is still undetermined. It has now been used in several thousand cases without accident.

Calcium Salts as a Preventative of Serum Sickness.

Netter (1906) has recently published a clinical study in which the internal administration of calcium chloride is advocated as a preventative of serum rashes. On the day of the injection and on the two following days he gives one gramme of calcium chloride. If more than 40 c.c. of serum are injected larger doses of the salt must be given and the treatment must be continued over a longer period. He claims to have had excellent results.

We have had absolutely no experience with this method.

THEORIES AS TO THE CAUSE OF SERUM SICKNESS.

Hamburger and Moro thought that the serum rashes were directly connected with precipitin formation, and in fact believed them to be due to precipitin formation in the capillaries. This view, however, was abandoned after Rostoski, Michaelis, and Oppenheimer showed that precipitin formation only took place in the test tube and did not occur in the living body. Further v. Pirquet and Schick (1905) showed that rashes often developed before the precipitins could be demonstrated, and, on the other hand, that precipitins may be abundant and yet no serum symptoms occur.

Nevertheless these authors believe that serum sickness is due to a chemical reaction between the horse serum and certain antibodies in the person injected. They are certain that these antibodies are not identical with the precipitins. They explain the long incubation period by regarding it as the interval necessary for the production of the antibody in response to the stimulus produced by the foreign serum. This antibody eventually reacts with some of the serum still present in the tissues. They point to the fact that a reinjection is associated with a shortened period of incubation which, according to them, is strong evidence of the correctness of the above explanation. The incubation period is lessened because the cells can now produce the antibody more quickly. In addition to this "accelerated reaction" v. Pirquet and Schick cite cases of "immediate reactions." Such an immediate reaction may be produced during a period not earlier than 12 days after the primary injection and not much later than about nine months after. During this time they believe the injected person contains some free antibody which, when brought into contact with the exciting serum (a reinjection), produces at once symptoms similar to those produced originally. If, however, the reinjection is performed sooner than the 12th day there is merely an "accelerated reaction," for up to that time no free antibody has been produced.

The immediate reaction seems to be comparable to the phenomenon described by Rosenau and Anderson (1906), and by Otto. These authors showed that very small injections of horse serum sufficed to make guinea-pigs extremely susceptible to subsequent injections of antitoxic or normal horse serum, provided the second injection was made 10 days or more after the first inoculation. Even feeding with horse meat sensitised guinea-pigs.

While guinea-pigs exhibit this phenomenon, other animals, and especially man, do not seem to be affected to the same extent. Children have in many instances been injected with anti-diphtheritic horse serum at intervals of two weeks without the production of serious symptoms. The few cases of sudden death on record have all occurred after the first injection of serum.

In many hundreds of hospital cases of which we have thorough knowledge a second immunising injection of antitoxin after an interval of two or three weeks has not produced more severe after-effects than the original injection. Some authors, however, have reported severe reactions with signs of collapse but no fatal cases.

SUDDEN DEATH AFTER AN INJECTION OF SERUM.

From time to time, but at long intervals, cases of sudden death after an injection of antitoxin have been reported. On closer investigation some of these clearly appear to be due to heart paralysis caused by the diphtheritic toxaemia. In a few cases death cannot be ascribed to this cause, and in a few others the cause cannot be considered as settled.

During 11 years over 50,000 persons in New York City have been injected with serum from the Department of Health and only two cases of sudden death after serum injection have been reported. In one family a two year old child suffering from diphtheria received 5000 units, and a healthy sister of six 1000 units. The inspector who had injected them left in about 10 minutes, as up to that time he had noticed no bad after-effects. A few minutes later, according to the mother, the immunised child suddenly collapsed and in a few moments was dead. An autopsy revealed the fact that the child had the status lymphaticus strongly marked. The sister, who received the larger injection from the same bottle, had no bad after-effects and made a good recovery.

The other case was that of a young lad, 16 years old, who was suffering from a well developed, but not malignant, pharyngeal diphtheria. He had been ill about 24 hours. He received the injection while in bed and did not seem to suffer from it at the moment. The physician and nurse left the room for a moment. They heard a peculiar sound and quickly stepped back into the room and found the boy dead. No autopsy was performed.

The following case of sudden collapse has also come to our knowledge.

A young man with a moderately severe attack of diphtheria was injected with about 5 c.c. of serum. In about two minutes he felt a tingling sensation and soon afterwards felt faint and as if sinking. Everything became black and he became unconscious. The physician found him cyanotic and with a very rapid heart action, and at once injected strychnine and carried out artificial respiration. In a few minutes the patient began to regain consciousness and in about half an hour was in the same condition as before the injection.

THE DURATION OF IMMUNITY.

It was early recognised that diphtheria antitoxin could be employed in immunising those exposed to diphtheria infection, and this use of the serum was advocated especially in France and in the United States. Thus in 1896 Biggs and Guerard were able to collect statistics dealing with over 17,000 persons injected with immunising doses, the great majority of whom had been in contact with diphtheria. Out of this large number only 131 were attacked by the disease, 110 within 30 days and 21 after that time. Of these 131 only two died.

Netter (1902) has collected the results of 34,350 immunising injections. In spite of the injections 206 (0·6%) of the persons developed diphtheria. Most of the observations deal with orphan asylums, schools, foundling asylums and the like.

Experience has proved that complete immunity is secured for only a short time. Four weeks is probably the average duration of immunity, but in some cases it is complete for only 10 to 14 days. At the Eleventh Congress for Hygiene and Demography, held at Brussels in 1903, practically all the observers agreed that passive immunity lasts in most cases for about three weeks, and that the injection of even large doses of serum is unable to greatly prolong this period. This fact is scarcely surprising when we remember that in the pre-antitoxin days second attacks of diphtheria were occasionally observed four to six weeks after the primary attack. Zuppinger (1904) has published a report on 1000 immunising injections in immediate relatives, nurses and others exposed to infection. Eighteen of the 1000 developed the disease within the first three or four weeks, but of these 11 showed symptoms within three days of the injection. These were probably already infected at the time of injection. The dose employed was 100 to 200 units.

Ibrahim has recently published a study on a large number of cases treated with antitoxin. Out of these he mentions eight in which second attacks occurred after 20, 21, 25, 31, 40, 41, 43, and 59 days respectively. Only such cases as developed typical whitish membrane on the throat and all the clinical symptoms of diphtheria were counted as relapses.

This point is important because so-called secondary membranes are frequently observed on the 8th—12th day. These are usually yellowish in colour and easily rubbed off and the patients lack the other clinical evidences of diphtheria.

In view of the short duration of both passive and active immunity it is advisable to repeat immunising injections every three weeks in persons exposed to diphtheria for a long time.

REPEATED INJECTIONS OF ANTITOXIN.

In view of the recent studies on immunity a number of observers have suggested that the injection of diphtheria antitoxin might be followed by the production of an antibody which would nullify the action of subsequent injections of antitoxin. Such a phenomenon is however never observed, and up to the present it has not been possible to produce an anti-antitoxin by injections of antitoxin. The injection of an antitoxic serum can, at the most, give rise to the development of a specific precipitin, which, in test tube experiments, causes a precipitate in the serum used for injection.

This precipitate carries down with it some of the antitoxin. Such a precipitation, however, does not occur in the body, and in the clinical use of antitoxic serum the precipitins are entirely without effect.

The effect of repeated injections in relation to serum sickness has already been discussed.

BIBLIOGRAPHY.

Throughout this book the Harvard System of references to the bibliography has been adopted. Whenever an author has been cited the date of publication has been added in brackets. In the following bibliography the names of the authors are given in alphabetical order, all the publications of a single author being included in one block. Following the date, which is printed in heavy type, is given the title of the paper and the name of the Journal, the latter being abbreviated in the manner adopted in the *Index Medicus*. The Roman numerals after the name of the Journal indicate the number of the volume, and the figures printed in ordinary type refer to the page. Thus :—Aaser (30. V. 1895), *Deutsche med. Wochenschr.*, XXI. 357, refers to the paper published on May 5th, 1895 on page 357 of the 21st volume of the *Deutsche medecinische Wochenschrift*. Unless otherwise stated the quotations are from the original papers. In those cases in which the original has been inaccessible, reference to a review of the paper has also been given.

Papers of purely clinical interest, which contain no references to the subjects which are considered in this book, have been omitted.

Aaser, P. (**30. V. 1895**), Zur Frage der Bedeutung des Auftretens der Loeffler'schen Diphtheriebacillen bei scheinbar gesunden Menschen. *Deutsche med. Wochenschr.*, XXI. 357.——(**1897**), Den bakteriologiske diagnose ved difteri. *Tidsskr. f. d. norske Laegefor.*, p. 21. (Ref. *Baumgarten's Jahresber.*, 1899, XIII. 322.)

Abbott, A. C. (**X. 1891**), Further studies upon the relation of the pseudo-diphtheria bacillus to the diphtheritic bacillus. *Johns Hopkins Hosp. Bull.*, II. 143.——(**13. V. 1893**), The etiology of membranous rhinitis (rhinitis fibrinosa). *Med. News*, 15 pp. repr.——(**1894**), The results of inoculations of milch cows with cultures of the bacillus diphtheriae. *Journ. Path. and Bact.*, II. 35–51.——(**17. XI. 1894**), A review of some of the disputed points in connection with diphtheria and conditions with which it has been confounded. *Med. News*, 16 pp. repr.——(**III. 1896**), The principles of preventive inoculation and serum therapeutics with considerations concerning their practical application. *Buffalo Med. Journ.*, 17 pp. repr.——(**1896**), The bacillus diphtheriae and some of the characters by which it may be identified. *Trans. Path. Soc. Philad.*, 1895–6, XVII. 253.——(**1899**), *Principles of Bacteriology*, 5th ed.——(**1902**), *The Principles of Bacteriology*, 6th ed., p. 400.

Abbott, A. C. and Ghriskey, A. A. (**IV. 1893**), A contribution to the pathology of experimental diphtheria, with special reference to the appearance of secondary foci in the internal organs. *Johns Hopkins Hosp. Bull.*, IV. 27.

Abbott, A. C. and Gildersleeve, N. (**18. XII. 1903**), On the branching occasionally exhibited by bacillus diphtheriae. *Centralbl. f. Bakteriol.*, Orig., XXXV. 273–280.

Abel, R. (**28. XII. 1892**), Zur Aetiologie der Rhinitis fibrinosa. *Centralbl. f. Bakt.*, xii. 841.——(**9. XII. 1893**), Beitrag zur Frage der Lebensdauer der Diphtheriebacillen. *Centralbl. f. Bakteriol.*, xiv. 756–761.——(**30. VIII. 1894**), Zur Kenntniss des Diphtheriebacillus. *Deutsche med. Wochenschr.*, xx. 692.——(**18. IX. 1894**), Ein Fall von Wunddiphtherie mit Nachweis von Diphtheriebacillen. *Centralbl. f. Bakt.*, xvi. 455.——(**29. XI. 1894**), Ueber die Schutzkraft des Blutserums von Diphtherieconvalescenten und gesunden Individuen gegen tödliche Dosen von Diphtheriebacillenculturen und Diphtheriebacillengift bei Meerschweinchen. *Deutsche med. Wochenschr.*, xx. 899.——(**10. I. 1895**), Ueber die Schutzkraft des Blutserums von Diphtheriekonvalescenten und gesunden Individuen gegen tödliche Dosen von Diphtheriebacillenkulturen und Diphtheriebacillengifte bei Meerschweinchen. *Ibid.* xvii. 36. [Original-referate.]——(**4. V. 1895**), Versuche über das Verhalten der Diphtheriebacillen gegen die Einwirkungder Winterkälte. *Ibid.* xvii. 545.——(**1897**), Der Diphtheriebacillus unter besonderer Berücksichtigung seiner Bedeutung für die Praxis. *Arch. f. Heilk.*, Wien. (Ref. *Centralbl. f. Bakt.*, 1897, xxii. 290.)

Adams, R. S. (**1894**), Klebs-Loeffler bacillus in healthy throats and in measles throats. *Med. Record*, N. Y., xlvi. 402.

Aetius (**1542**), *Aëtii medici graeci contractae ex veteribus medicinae tetrabiblos.* Froben Basiliae.

Affleck, J. O. (**1877**), Diphtheria. *Encyclop. Britann.*, vii. 249, 9th ed.

Alaymus (**1632**), *Consultatio pro ulceris syriaci nunc vagantis curatione.* Panormi.

Albers, J. A. (**1816**), *Commentatio de tracheitide infantum, vulgo Croup vocata.* Lipsiae.

Albu, A. (**1897**), Untersuchungen über die Toxicität normaler und pathologischer Serum-flüssigkeiten. *Virchow's Archiv*, cxlix. 405.

Alessandro (**1898**), Morfologia del bacillo der Xerosis epiteliale. *Policlinico*, No. 15. (Ref. *Centralbl. f. innere Med.*, 1899, xx. 809.)

Allen, M. K. (**15. IV. 1902**), Diphtheria. *American Pract. and News.*

Allen, S. (**13. X. 1900**), The present status of our knowledge concerning the bacteriology and serum treatment of diphtheria. *Journ. Amer. Med. Assoc.*

Althaus, J. (**1860**), Die Diphtheritis in England. *Wien. med. Wochenschr*, x. 245.

Anderson, J. F. (**1906**), Transmission of resistance to diphtheria toxine by the female guinea-pig to her young. *Journ. of Med. Research*, xv. 241.——(**1906**), Simultaneous transmission of resistance to diphtheria toxine and hyper-susceptibility to horse serum by the female guinea-pig to her young. *Ibid.* xv. 259.

Andrade, E. (**III. 1904**), A New Stain for Diphtheria Bacilli. *Med. News*, lxxxiv. 498.——(**XII. 1904**), Some remarks on diphtheria bacilli. *Ibid.* lxxxv. 1069.

Andrewes, F. W. (**1893**), Preliminary report on the growth of bacteria in pus. *Local Government Board Report* for 1891–2, p. 209.——(**1896**), On the occurrence of the diphtheria bacillus in a series of cases of sore throat amongst the female staff of the hospital. *St Barth's. Hosp. Rep.*, xxxii. 145.——(**29. IX. 1900**), (Distribution of diphtheria bacilli.) *Brit. Med. Journ.*, ii. 907. (Report of Meeting of Brit. Med. Ass. at Ipswich, 1. VIII. 1900.)

Appleget, F. B. (**26. VIII. 1893**), Twenty-eight cases of diphtheria with eleven deaths, due to infected milk supply. *Philad. Med. News*, lxiii. 238.

Arloing, F. (**1895**), Action du sérum normal et antidiphtérique sur l'organisme. *Lyon Med.*, lxxix. 151.——(**1899**), Influence de la voie d'introduction sur le développement des effets thérapeutiques du sérum antidiphtérique. *Comptes Rendus de l'Acad. d..Sciences*, cxxviii. 1, 498.——(**21. XI. 1901**), Influence de la mucine sur le bacille de Loeffler et sur sa toxine. *Compt. rend. Soc. de Biol.*, LIII. 1117.——(**1902**), Recherches sur le pouvoir bactéricide de la mucine. *Journ. de phys. et path. générale*, IV. 291. (Ref. *Centralbl. f. Bakt.*, 1903, XXXIII. 547.)

Arloing, S. and Nicolas, J. (**1901**), Nouveaux essais sur la production rapide de l'antitoxine diphtérique par l'association du sérum antidiphtérique à la toxine. *Compt. rend. Soc. de Biol.*, LIII. 36, also *Journ. de physiol. et de pathol. génér.*, III.

Arloing, S., Nicolas, J. and Antoine, G. (**1901**), Essais sur la production rapide de l'immunité et de l'antitoxine diphtériques. *Compt. rend. Soc. de Biol.*, LIII. 13.

Armstrong, H. E. (**1906**), Diphtheria, its extent and fatality in the large provincial towns of England and Wales, and in the hospitals of the Metropolitan Asylums Board, during the years 1894–1904 inclusive, in relation to the use of antitoxin serum in the treatment of the disease. *Pub. Health*, XVIII. 227.

Arndt (**1896**), Die Technik der bacteriologischen Diagnose der Diphtherie. *Correspdzbl. f. schweizer Aerzte*, No. 20, p. 640. (Ref. *Baumgarten's Jahresber.*, 1898, XII. 222.)

Arnheim, G. (**1891**), Anatomische Untersuchungen über diphtherische Lähmungen. *Arch. f. Kinderheilk.*, XIII. 461–467.

Aronson, H. (**1892**), Ueber die antiseptischen Eigenschaften des Formaldehyds. *Berlin. klin. Wochenschr.*, 749.——(**1893**), Experimentelle Untersuchungen über Diphtherie und die immunisierende Substanz des Blutserums. *Ibid.* Nos. 25–26. (Ref. *Centralbl. f. Bact.*, XIV. 260 (Voges).)——(**1893**), Recherches expérimentales sur la diphtérie et sur la substance immunisante du sérum. *La Semaine Méd.*, XIII. 284. (Rept. Berlin Med. Soc., 31. V. 1893.)——(**1894**), Weitere Untersuchungen über Diphtherie und das Diphtherie-Antitoxin. I. Ueber die Art und Weise der Antitoxinwirkung. *Berlin. klin. Wochenschr.*, 426. (Ref. *Centralbl. f. Bakt.*, XV. 926 (Gerlach).)——(**1894**), Meine Stellung in der Diphtherie-antitoxinfrage. *Berlin. klin. Wochenschr.*, 1026, 1077.——(**1900**), Zur Biologie und Chemie der Diphtheriebacillen. *Arch. f. Kinderheilk.*, XXX. 23.——(**1902**), Zur Biologie und Chemie der Diphtheriebacillen. *Arch. f. Kinderheilk.*, XXX. 23. (Ref. *Baumgarten's Jahresber.*, 1904, XVIII. 220.)

Arrhenius, S. (**1904**), Anwendung der physikalischen Chemie auf die Serumtherapie. Berlin, 1904. *Arb. a. d. K. Gesundheitsamte*, XX.——(**1907**), *Immunochemie.* Anwendung des physikalischen Chemie auf die Lehre von den physiologischen Antikörpern. Akad. Verlagsgesellschaft: Leipzig.

Arrhenius, S. and Madsen, T. (**1902**), Physical Chemistry applied to Toxins and Antitoxins. *Contrib. from the Univ. Lab. for Med. Bacteriol.*, to celebrate the Inauguration of the Serum Inst., Paper No. III., 17 tables, 7 figs., pp. 3–87. Copenhagen : O. C. Olsen and Co.——(**1902**), The molecular weight of diphtheria toxin. *Ibid.*, Paper No. IV., pp. 1–7.——(**1903**), Anwendung der physikalischen Chemie auf das Studium der Toxine und Antitoxine. *Zeitschr. f. physikal. Chem.*, XLIV. 7.——(**1904**), Toxines et Antitoxines. Le poison diphtérique. *Acad. roy. d. sci. et lettres de Danemark.* No. 4 (reprint).

d'Arsonval, M. and Charrin, A. (**1896**), Action des diverses modalités éléctriques sur les toxines bactériennes, etc. *Compt. rend. Soc. Biol.*, 96, 121, 153. (Ref. *Baumgarten's Jahresber.*, XII. 228, 710.)

Arthus (**1903**), Injections répétées de sérum de cheval chez le lapin. *Compt. rend. Soc. de Biol.*, 817.

Asakawa, N. (**1898**), Die Basis der natürlichen Immunität des Huhns gegen Tetanus. *Centralbl. f. Bakt.*, XXIV. 166, 234.

Ascher and Symanski (**1898**), Bakteriologische Erfahrungen über die Königsberger Thierlymphe. *Zeitschr. f. Hyg.*, XXVIII. 335.

Ashby, A. (**XII. 1906**), A milk epidemic of diphtheria associated with an udder disease of cows. *Public Health*, XIX. 145.

Ashby, H. (**19. III. 1892**), The bacteriological diagnosis of diphtheria. *Lancet*, I. 664. (Letter.)

Astros, L. and Rietsch, M. (**1900**), Essais d'extraction de l'antitoxine diphtéritique *Compt. rend. Soc. de Biol.*, LII. 337.

Atkinson, J. P. (**1899**), A preliminary note on the fractional precipitation of the globulin and albumin of normal horse's serum and diphtheric antitoxic serum, and the antitoxic strength of the precipitates. *Journ. Exper. Med.*, IV. 649. ——(**1900**), The fractional precipitation of the globulin and the albumin of normal horse serum and diphtheria antitoxic serum, and the antitoxic strength of the precipitates. *Ibid.* V. 67.——(**1903**), The period of development, the time of greatest accumulation, and the persistence of diphtheria antitoxin in the blood of a series of one hundred horses. *Journ. Med. Research*, IX. 173. ——(**1903**), Precipitins formed by injecting animals with globulin. *Proc. New York Path. Soc.*, 193. (Ref. *Biochem. Centralbl.*, II. 627, 1904.)

Atlasow, J. (**X. 1902**), *Wojenno-med. Shurnal.* (Ref. *Baumgarten's Jahresber.* 1904, XVIII. 203.)

Auckenthaler (**16. IV. 1898**), Beitrag zur Diagnose des Diphtheriebacillus. *Centralbl. f. Bakt.*, XXIII. 641.

Auden, G. A. (**15. IV. 1902**), Diphtheria in the newly born. *Lancet*, I. 1104.

Ausiaux, G. (**1895**), Pseudo-diphtérie à streptocoques; bronchopneumonie; mort. *Bull. Acad. roy. de méd. de Belg.*, IX. 82.

Autenrieth, J. F. H. (**1807**), Heilmethode bey der häutigen Luftröhrenentzündung der Kinder. *Versuche f. d. prakt. Heilk. a. d. klin. Anstalten von Tübingen*, I. (1), 9.

Axenfeld, T. (**28. II. 1898**), Wie weit sind die sogenannten Xerosebacillen der Conjunctiva mit den Hofmann-Loeffler'schen Pseudodiphtheriebacillen des Rachens identisch? *Berlin. klin. Wochenschr.*, XXXV. 189.——(**13. VI. 1898**), Nochmals: das Verhältniss der sog. Xerosebacillen der Conjunctiva zu den Hofmann-Loeffler'schen Pseudodiphtheriebacillen des Rachens. *Ibid.* XXXV. 542.——(**8. IV. 1902**), Zu dem Aufsatze von Schanz, Zu Behring's neuester Diphtherietheorie. *München. med. Wochenschr.*, XLIX. 580.

Azerno-Schwarz (**1901**), Die Bedeutung der bacteriologischen Methode für die Diphtheriediagnose. *Arch. f. Kinderheilk.*, XXXIX. 113.

Babbe and Bartels, Krankheiten des Harnapparates in *v. Ziemssen's Handb.*, IX. 1.

Babes, V. (**20. II. 1886**), Les Spores des bacilles de la diphtérie humaine. *Progrès méd.*, III. (Ref. *Baumgarten's Jahresber.*, II. 273.)——(**1889**), Lésions de la diphtérie expérimentale de l'homme et des pigeons. *Ann. de l'Inst. de Pathol. et de Bactériol. de Bucarest*, I. 310. (Ref. *Baumgarten's Jahresber.*, VII. 230.)——(**1889**), Ueber isolirt färbbare Antheile von Bacterien. *Zeitschr. f. Hyg.*, V. 173; also *Progrès méd.*, 1889.——(**1890**), Untersuchungen über den Diphtheriebacillus und die experimentelle Diphtherie. *Arch. f. path. Anat.*, CXIX. 460.——(**1895**), Beobachtungen über die metachromatischen Körperchen, Sporenbildung, Kolben- und Kapselbildung pathogener Bakterien. *Zeitschr. f. Hyg.*, XX. 412.——(**10. II. 1899**), Ueber die Kultur der von mir bei Lepra gefundenen Diphtheridee. *Centralbl. f. Bakt.*, XXV. 125

Babes, V. and Lepp (**1889**), Recherches sur le vaccination antirabique. *Fortschr. d. Med.*, 485. (Ref. *Baumgarten's Jahresber.*, V. 135.)

Babes, V. and Puscariu, E. (**1890**), Untersuchungen über die Diphtherie der Tauben. *Zeitschr. f. Hyg.*, VIII. 376–403.

Baginsky, A. (**1890**), Zur Aetiologie der Diphtherie. *Arch. f. Kinderheilk.*, XIV. (Ref. *Centralbl. f. Bakt.*, X. 806.)——(**1892**), *Berlin. klin. Wochenschr.*, No. 9.——(**1892**), Zur Aetiologie der Diphtheritis. *Deutsche med. Wochenschr.*——(**1893**), Die klinischen Erscheinungen der diphtherischen Nierenerkrankung.

Arch. f. Kinderheilk., xvi.——(1895), *Die Serumtherapie bei Diphtherie.* 330 pp. 8⁰. Berlin: Hirschwald.——(1898), "Diphtheria" in *Nothnagel's Specielle Pathol. u. Therapie*, ii. Vienna.——(1898), *Diphtherie und diphtheritischer Croup.* Wien.——(1899), Article "Diphtheria" in *Nothnagel's Spec. Pathol. u. Therap.*, ii. Vienna.

Baginsky, A. and Sommerfeld, P. (**2. VII. 1900**), Ueber einen constanten Bakterienbefund bei Scharlach. *Berlin. klin. Woch.*, xxxvii. 588, 618.

Baldassari, L. (**1896**), Ueber die Wirkung der Diphtherietoxine auf den Zellkern. *Centralbl. f. allg. Path.*, vii. 625–630.

Baldwin, E. R. and Levene, R. A. (**1901**), The action of proteolytic enzymes on bacterial toxines. *Journ. of Med. Research*, vi. 120.

Ballin, L. (**1903**), Ueber das Vorkommen von Diphtheriebazillen beim gewöhnlichen Schnupfen der Säuglinge. *Jahrb. f. Kinderheilk.*, lviii. 412.

Ballonius, G. (**1762**), *Opera medica omnia.* Geneva.

Bandi, I. (**1903**), Ueber die Bereitung eines antibacteriellen Diphtherieserums, sein prophylaktischer und Heilwert. *Centralbl. f. Bakt.*, 1 Abt., xxxiii. 535.

Bandi, I. and Gagnoni, E. (**1906**), Die Vaccination gegen Diphtherie. *Centralbl. f. Bakt.*, 1 Abt., xli. 386.

Bang, B. (**17. II. 1893**), Om Aarsagen til lokal Nekrose. *Maanedskr. f. Dyrloeger*, ii. 235. (Ref. *Centralbl. f. Bakteriol.*, xiii. 201.)

Banzhaf and Gibson (**1907**), The Fractional Precipitation of Antitoxic Serum. *Journ. Biol. Chem.*, iii. 253.

Barannikow (**1899**), Zur Frage über die Bakteriologie der Lepromata. *Centralbl. f. Bakt.*, xxvi. 113.

Barbacci, O. (**1896**), Ueber die feineren histologischen Alterationen der Milz, der Lymphdrüsen, und der Leber bei der Diphtherie-Infection. *Centralbl. f. allg. Pathol.*, vii. 321–358.

Barbier, A. (**1891**), De la transmissibilité de la diphthérie aviaire à l'homme. *Ann. de la Soc. médico-chirurgicale de Liège.* (Ref. *Centralbl. f. Bakt.*, 1891, x. 93.)

Barbier, H. (**1889**), De l'importance des fumiers et des oiseaux de basse-cour dans l'étiologie de la diphtérie. *Gaz. méd. de Paris*, vi. 37.——(**1891**), De quelques associations microbiennes dans la diphtérie. *Arch. de méd. expér. et d'anat. path.*, ii. (Ref. *Centralbl. f. Bakt.*, 1892, xi. 382–384. Escherich.)——(**1892**), Sur un streptocoque particulier trouvé dans les angines à fausses membranes, seul ou associé au bacille de la diphtérie (diplostreptocoque). *Arch. de méd. expér. et d'anat. path.*, iv. 827.——(**21. VI. 1893**), Sur un mode d'infection septique par le streptocoque dans la diphtérie. *Compt. rend. Soc. de biol.*, Sér. IX., v. 672.——(**1897**), Méthodes bactériologiques et cliniques dans le diagnostic de la diphtérie. *Bull. et mém. Soc. méd. de hôp. de Paris*, xiv. 956.——(**1897**), Recherches bactériologiques chez les diphtériques; présence du bacillus dans les viscères. *La Semaine Méd.*, xvii. 411. (Report of Meeting of Soc. méd. des Hôpitaux, 29. X. 1897.)——(**1898**), Nouvelles recherches sur les formes cliniques et bactériologiques de la diphtérie. *Bull. et mém. Soc. Méd. d. Hôp. de Par.*, xv. 45.——(**1898**), De la valeur du bacille court pour le diagnostic de la diphtérie. *La Semaine Méd.*, xviii. 460. (Report of Meeting, 11. XI. 1898, of Soc. Méd. des Hôpitaux.)——(**1904**), Accidents sérothérapique ou pseudohumatisme diphtérique. *Bull. de la Soc. Pediat., Paris*, vi.

Barbier, H. and Ulmann, G. (**1899**), *Les actualités médicales.* La diphtérie. Nouvelles recherches bactériologiques et cliniques; prophylaxie et traitement. Paris.

Bard, S. (**1789**), *Trans. American Phil. Soc.*, Philadelphia, I.

Bardach, J. (**I. 1895**), Études sur la diphtérie. *Ann. Inst. Pasteur*, ix. 40.

Barlow, H. W. L. (**3. XII. 1898**), Diphtheria bacilli in urine. *Lancet*, ii. 1471.

Barnes, E. G. (**28. VII. 1888**), An address on the etiology of diphtheria. *Brit. Med. Journ.*, ii. 167.

Barranikow, J. (**1901**), Russki shurnal koshnych i weneriezeskych bolenjei. (Ref. *Baumgarten's Jahresber.*, xvii. 166.)

Bartel, T. (**22. IX. 1898**), Ueber den Bakteriengehalt der Luftwege. *Centralbl. f. Bakt.*, xxiv. 401.

Bass (**1894**), Ueber eine Ophthalmia hepatitica. *von Graeffe's Arch. f. Ophthal.*, xl. 608, Abt. 5. (Ref. *Centralbl. f. Bakt.*, 1895, xvii. 840.)

Bauer, J. (**1906**), Ueber den Befund von Diphtheriebacillen in faulen Mundecken und Panaritien. *Arch. f. Kinderheilk.*, lxxiv. 55.

Baumgarten, P. (**1897**), Untersuchungen über die Pathogenese und Aetiologie der diphtherischen Membranen. *Berlin. klin. Wochenschr.*, xxxiv. 665, 691.

Baurowiez, A. (**1895**), *Przeglad lekarski*, Nos. 46, 47, 48. (Autoreferat. *Centralbl. f. Bakt.*, xviii. 719.)

Bayne, F. C. (**V. 1902**), Bacteriological diagnosis of membranous inflammation of the throat by a simple method. *Journ. of Eye, Ear, and Throat Dis.*, vii. (Ref. *Baumgarten's Jahresber.*, 1904, xviii. 206.)

Beaton, R. M., Caijer, F. F. and Pakes, W. C. C. (**21. IX. 1901**), The value of Neisser's stain in the diagnosis of diphtheria. *Brit. Med. Journ.*, ii. 758.

Beck, A. and Slapa, W. (**1895**), Ueber den Einfluss des Diphtheriegiftes auf den Kreislauf. *Wien. klin. Wochenschr.*, viii. 321.

Beck, M. (**1890**), Bacteriologische Untersuchungen über die Aetiologie der menschlichen Diphtherie. *Zeitschr. f. Hyg.*, viii. 434.——(**1901**), Ueber die desinficirenden Eigenschaften der Peroxole. *Ibid.* xxxvii. 294–306.

Beco, L. (**1896**), Contribution à l'étude de la stomatite diphtéroide infantile. *Arch. de méd. expér.*, viii. (Ref. *Hyg. Rundsch.*, 1896, vi. 995.)

Behrens, B. (**1901**), Einfluss der Witterung auf Diphtherie, Scharlach, Masern und Typhus. *Arch. f. Hyg.*, xl. 1.

Behring, E. (**1888**), Ueber die Ursache der Immunität von weissen Ratten gegen Milzbrand. *Centrabl. f. klin. Med.*——(**1890**), Untersuchungen über das Zustandekommen der Diphtherie-Immunität bei Tieren. *Deutsche med. Wochenschr.*, xvi. 1145.——(**24. XII. 1891**), Ueber Desinfektion am lebenden Organismus. *Ibid.* xvii. 1393.——(**1891**), Zur Immunitätsfrage. *Deutsche med. Wochenschr.*, xvii. 655.——(**1892**), *Die Blutserumtherapie I.* Leipzig, 1892. ——(**1892**), Die Blutserumtherapie bei Diphtherie u. Tetanus. *Zeitschr. f. Hygiene*, xii. 1.——(**1893**), *Die Geschichte der Diphtherie, mit besonderer Berücksichtigung der Immunitätslehre.* Leipzig.——(**1893**), *Gesammelte Abhandlungen zur aetiologischen Therapie von ansteckenden Krankheiten.* G. Thieme, Leipzig, 1893.——(**1893**), Ueber den gegenwärtigen Stand der Blutserumtherapie. *Arch. f. Physiologie*, 198.——(**1893**), Ueber die Natur der immunitätverleihenden Körper. *Arch. f. Phys.*, 381.——(**1893**), Ueber sogenannte septische Fälle von Diphtherie. *Deutsche med. Wochenschr.*, xix. 543.——(**1894**), *Infektion und Desinfektion. Bekämpf. d. Infektionskrankh.* Leipzig. ——(**20. X. 1898**), Thatsächliches, historisches und theoretisches aus der Lehre von der Giftimmunität. *Deutsche med. Wochenschr.*, xxiv. 654.——(**1899**), Ueber die Beziehungen der Blutantitoxine zu den zugehörigen Infectionsgiften. *Ibid.* xxv. 3.——(**1899**), Ueber Tetanusgiftmodificationen. *Ibid.* 501.——(**1899**), Ueber die quantitativen Bindungsverhältnisse zwischen Tetanusgift und Tetanusantitoxin im lebenden Meerschweinkörper. *Fortschr. d. Med.*, 521. ——(**1899**), Allg. Therapie der Infektionskrankheiten; aus d. *Lehrbuch der allg. Therapie* von Eulenburg u. Samuel. Urban und Schwarzenberg, Berlin und Wien, 1899.——(**1901**), *Diphtherie.* Bibliothek Coler. 8^0, 185 pp. Hirschwald: Berlin.——(**1902**), Die Serumtherapie in der Heilkunde in Heilkunst. *Nordiskt Med. Ark.*, xxxiv. A. and D. 11 Häft. p. 3.

Behring, Boer and Kitasato (**1893**), Zur Behandlung diphtheriekranker Menschen mit Diphtherieheilserum. *Deutsche med. Wochenschr.*, xix. 389, 415.

Behring and Kitasato (1890), Ueber das Zustandekommen der Diphtherieimmunität und die Tetanusimmunität bei Tieren. *Deutsche med. Wochenschr.*, xvi. 1113.

Behring and Nissen (1890), Ueber bacterienfeindliche Eigenschaften verschiedener Blutserumarten. Ein Beitrag zur Immunitätsfrage. *Zeitschr. f. Hygiene*, viii. 412.

Behring, E. and Wernicke (1892), Ueber Immunisierung und Heilung von Versuchsthieren bei der Diphtherie. *Zeitschr. f. Hyg.*, xii. 10.

Beitzke, H. (26. V. 1904), Ueber die fusiformen Bacillen. *Centralbl. f. Bakt.*, xxxv. 1.

Belfanti, S. (1894), Sulla propagazione del virus difterico. *La Rif. med.*, No. 69. (Ref. *Centralbl. f. Bakt.*, xvi. 790.)——(1895), Sulle broncopolmoniti difteriche; studio batteriologico ed anatomopatologico. *Sperimentale*, Sez. biol., Firenze, xlix. 278–299.

Belfanti, S. and Carbone, T. (1898), Contributo alla conoscenza dell' antitossina difterica. *Arch. per le Sci. med.*, xxiii. (Ref. *Centralbl. f. Bakt.*, xxiii. 906.)

Bendix (1903), "Diphtheria" in *Lehrbuch d. Kinderheilkunde*, 3rd ed. Berlin.

Benham, C. H. (5. V. 1906), The bacteriology of a common cold. *Brit. Med. Journ.*, i. 1023.

Bennet, Ch. (1665), *Tabidorum theatrum, sive phtisios, atrophicæ et hecticæ Xenodochium*, pp. 55, 78. London.

Bennett (9. X. 1842), On abnormal nutrition in softening, suppuration, etc. Abstract of a communication made to the Med. Chir. Soc. Edinburgh. (Ref. *Zeitschr. f. rat. Med.*, ii. 1844, 326.)

Bensawde, R. (1897), *Le phénomène de l'agglutination des microbes et les applications à la pathologie.* Paris, 1897. Charre et Naud.

Bergen, van (1764), *Nova acta naturae curiosorum*, ii. 157. Francof. et Lipsiae.

Bergey, D. H. (1898), Comparative studies upon the pseudo-diphtheria or Hofmann bacillus, the Xerosis bacillus and the Löffler bacillus. *Publications of the University of Pennsylvania*, New Series, No. 4.——(1904), The occurrence of bacillus pseudodiphtheriae in cow's milk. *Journ. Med. Research*, xi. 445.

Berkley, H. J. (II. 1897), Lesions induced by the action of certain poisons on the nerve cells. Diphtheria. *Johns Hopkins Hosp. Bull.*, viii. 23.

Bernard, L. and Felsenthal, S. (1893), Beitrag zur pathologischen Anatomie der Diphtherieniere. *Arch. f. Kinderheilk.*, xvi. 308.

Bernard, R. and Jacob, O. (IX. 1903), Gangrène cutanée diphtérique. *Arch. de Méd. expér. et d'anat. pathol.*, xv. 685.

Bernardbeig (1894), *Complications articulaires de la diphtérie.* Thèse, Paris, 1894.

Bernheim, J. (1894), Ueber die Mischinfection bei Diphtherie. *Zeitschr. f. Hyg.*, xviii. 529.——(1897), Ueber die Rolle der Streptococcen bei der experimentellen Mischinfection mit Diphtheriebacillen. *Arch. f. Hyg.*, xxviii. 138.——(1898), Ueber Immunisirung von Versuchsthieren gegen die Mischinfection mit Diphtheriebacillen und Streptococcen. *Ibid.* xxxiii. 35.

Bernheim, J. and Folger, C. (25. VII. 1896), Ueber verzweigte Diphtheriebacillen. *Centralbl. f. Bakt.*, xx. 1.——(19. XII. 1896), Ueber verzweigte Diphtheriebacillen. Eine Antwort an Herrn Dr A. A. Kanthack. *Ibid.* xx. 905.

Berry, F. H. (1900), Advantages of bacteriological diagnosis as instanced by the history of an outbreak of diphtheria in a large school. *Trans. Epidem. Soc.*, xix. 117.

Berry, J. L. (12. II. 1898), An epidemic of diphtheria; demonstrating, in a marked degree, its contagious nature, and the value of immunisation. *Med. Record* (New York), liii. 864. (Ref. *Hyg. Rundsch.*, ix. 1200.) [Nuttall.]

Besredka (**V. 1896**), De la leucocytose dans la diphtérie, étude expérimentale et clinique. *Ann. Inst. Pasteur*, xii. 305–342.

Besser, L. v. (**1889**), Ueber die Bakterien der normalen Luftwege. *Ziegler's Beiträge*, vi. 331.——(**4. V. 1893**), Ein noch nicht beschriebener Bacillus bei der Variola vera. *Centralbl. f. Bakt.*, xiii. 590.

Bettmann, H. W. (**6. VI. 1895**), Diphtheria; its bacterial diagnosis and treatment with the antitoxin. *Philad. Med. News*, lxvii. 1.

Bezancon, F. (**1895**), De la rate dans la diphthérie. *Rev. mens. d. mal. d. l'enf.*, Par. xiii. 405–412.

Bezancon, F. and Labbé, M. (**7. V. 1898**), Effets comparés de l'action sur les ganglions du bacille et de la toxine diphtérique. *Compt. rend. Soc. de Biol.*, Series X., v. 507.

Biernacki, J. and Heanley, C. M. (**1906**), Discharge from the ear as a probable factor in the spread of diphtheria. *Journ. Prevent. Med.*, xiv. 423.

Biggs, C. P. (**27. X. 1900**), Diphtheria and milk supplies. *Philad. Med. Journ.*, 771. (Report of New York State Med. Soc. Meeting, 15. X. 1900.)

Biggs, G. (**1893**), *Proc. New York Path. Soc.* (Quoted by Salmon, 5. III. 1904.)——(**1894**), Diphtheritic vaginitis. *Proc. N. Y. Path. Soc.*, p. 44.

Biggs, H. M. (**1893**), Bacteriological examinations made in diphtheria by the health department. *Med. Rec.*, N. Y., xliv. 695.——(**1894**), Report to the New York City Health Department on the use of bacteriological examination for the diagnosis of diphtheria. *Med. Rec.*, N. Y., xlvi. 321.——(**X. 1896**), Some investigations as to the virulence of the diphtheria bacilli occasionally found in the throat-secretions in cases presenting the clinical features of simple acute angina. *Amer. Journ. Med. Sci.*, cxii. 411.

Biggs and Guerard (**1896**), The use of antitoxin serum in the treatment of diphtheria, etc. *Med. News*, N. Y., lxix.

Biggs, H. M., Park, W. H. and Beebe, A. L. (**1895**), Report on bacteriological investigations and diagnosis of diphtheria from May 4th, 1893 to May 4th, 1894. *Scientific Bulletin*, No. 1, *Health Department*, *City of New York.*

Bilhaut (**1890**), Propagation de la diphtérie du pigeon à l'homme; invasion consécutive du même mal chez un enfant. *Bull. Soc. de méd. prat. de Par.*, 710.

Billings, J. S. (**1896**), The blood corpuscles in diphtheria with special reference to the effect produced upon them by the antitoxin of diphtheria. *Med. Record*, N. Y., lxix.——(**1903**), The value of confirmatory cultures in diphtheria, based on the work of the Diagnosis Laboratory of the Dept. of Health of the City of New York. *New York Med. Journ.*, lxxviii. 493.——(**1905**), Ten years' experience with Diphtheria Antitoxin. *Ibid.* lxxxii.

Billington and O'Dwyer (**1889**), *Diphtheria and Croup.* 326 pp., 8⁰. New York.

Billroth, T. (**1870**), Ueber die Beziehung der Rachen-Diphtherie zur Septämie und Pyohämie, mit Nachtrag. *Wien. med. Wochenschr.*, xx. 105.

Bing and Ellerman (**1904**), Uber Diphtherie Statistik. *Therap. Monatsch.*, xviii.

Birch-Hirschfeld, F. V. (**1878–9**), Ueber plötzliche Todesfälle nach Diphtherie. *Jahresber. d. Ges. f. Natur. u. Heilk.*, Dresden, 26–31.

Bissell, W. G. (**2. II. 1895**), Bacteriologic examination in the diagnosis of diphtheria. *Philad. Med. News*, lxvi. 122.——(**1895–6**), Laboratory experience in the bacteriology of diphtheria. *Buffalo Med. Journ.*, xxxv. 626.——(**XII. 1900**), Three varieties of membranous anginas produced by microorganisms other than the Klebs-Loeffler bacillus and their sanitary significance. *Buffalo Med. Journ.*, p. 312. (Ref. *Baumgarten's Jahresbericht*, xvi. 170.)——(**31. V. 1902**), The bacterial pathology, symptomatology diagnosis, treatment and quarantine of tonsilar inflammations. *Med. News*, lxxx. 1012–1016.

Bisson, A. O. (**1906**), The injection of antitoxin in diphtheria by the intravenous method. *Lancet*, ii. 929.

Bizzozero (1876), Beiträge zur pathologischen Anatomie der Diphtheritis. *Med. Jahrbuch*, Wien.——(1885), Ueber das constante Vorkommen von Bacterien in den Lymphfollikeln des Kaninchens. *Centralbl. f. d. med. Wiss.*, xxiii. 801.

Blackburn, A. E. (14. V. 1904), Sore throats which present symptoms and physical signs of diphtheria. *Amer. Med.*, vii. 772.

Blakely, D. N. and Burrows, F. G. (25. VII. 1901), Diphtheria as a complication of measles. *Boston Med. a. Surg. Journ.*, cxlv.

Blandi, I. (1903), Ueber die Bereitung eines antibakteriellen Diphtherieserum. *Centralbl. f. Bakt.*, xxxiii. 535.

Blasi, L. and Russo-Travali, G. (VII. 1896), Contribution à l'étude des associations bactériennes dans la diphtérie. *Ann. de l'Inst. Pasteur*, x. 387.

Bloch, W. and Sommerfeld, P. (1900), Beiträge zur Pathogenität des Loeffler-Bacillus. *Archiv. f. Kinderheilk.*, xxviii. 40. (Ref. *Baumgarten's Jahresber.*, xvi. 195.)

Blumenthal, A. (1898), Beitrag zum Verhalten des Diphtheriebacillus auf künstlichen Nährböden und im thierischen Organismus. *Zeitschr. f. klin. Med.*, xxxv. 573.

Blumenthal, F. (1897), Ueber die Möglichkeit der Bildung von Diphtherietoxin aus Eiweisskörpern und auf Zucker enthaltenden Nährboden. *Deutsche med. Wochenschr.*, xxiii. 382.

Blumenthal, J. M. and Lipskerow, M. (1905), Vergleichende Bewertung der differentiellen Methode zur Färbung des Diphtheriebacillus. *Centralbl. f. Bakt.*, xxxviii. 359.

Boarsohn (1895), *Zur Aetiologie der Bronchopneumonie bei Diphtherie.* Dissertation. Freiburg.

Boer, O. (1891), Ueber die Behandlung diphtherieinficirter Meerschweinchen mit chemischen Präparaten. *Zeitschr. f. Hyg.*, xi. 154-164.

Bokay, v. (1895), Meine Erfolge mit Behring's Diphtherieheilserum. *Deutsche med. Woch.*, xxi.

Bolton, A. (1905), Primary heart failure as the immediate cause of death in acute diphtheritic toxaemia. *Lancet*, i. 278.

Bolton, B. M. (1896), Diphtheria antitoxin sometimes found in the blood of horses that have not been injected with toxin. *Journ. Exper. Med.*, i. 543.——(1896), The examination of cultures from cases of suspected diphtheria. *Med. and Surg. Reporter*, Philad., lxxiv. 799.

Bolton, B. M. and Pease, H. D. (1896), The production of antitoxin by the passage of electricity through diphtheria cultures. *Journ. of Exper. Med.*, i. 537.

Bolton, C. and Brewer, D. (29. IV. 1905), A case of extensive cutaneous diphtheria with an examination of the nervous system. *Lancet*, i. 1130.

Bomstein (1897), Zur Frage der passiven Immunität bei Diphtherie. *Centralbl. f. Bakt.*, xxii. 587.——(1898), Ueber das Schicksal des Diphtherietoxins im Thierorganisms. *Centralbl. f. Bakt.*, xxiii. 785.

Bonain (1895), *Rev. de Laryngol. d'Otolog., et de Rhinol.*, No. 9 (quoted in *Amer. Journ. Med. Sci.*, VI. 1895, cx. 239).

Bond, F. T. (17. I. 1898), A source of diphtherial infection. *Brit. Med. Journ.*, i. 19. ——(1906), On some points of interest in the treatment of outbreaks of diphtheria. *Journ. Roy. San. Inst.*, xxvii. 388.

Bonhoff (1. II. 1896), Ueber die Wirkung der Streptokokken auf Diphtheriekulturen. *Hyg. Rundsch.*, vi. 97.

Boni, I. (8. XII. 1900), Methode zur Darstellung einer "Kapsel" bei allen Bacterienarten. *Centralbl. f. Bakt.*, xxviii. 705.

Booker, W. D. (X. 1892), The relation of pseudo-diphtheritic angina to diphtheria, with special reference to scarlatinal pseudo-membranous angina. *Johns Hopkins Hosp. Bull.*, iii. 109.——(1893), As to the aetiology of primary pseudomembranous inflammation of the larynx and trachea, with remarks on the distribution of diphtheria bacilli in organs of the body distant from the seat of local infection. *Arch. Paediatrics*, N. Y., x. 642–652.

Bordet, J. (1900), Les sérums hémolytiques, leurs antitoxines et les théories des sérum cytolytiques. *Ann. Inst. Pasteur*, xiv. 257.——(1901), Sur la mode d'action des sérums cytolytiques et sur l'unité de l'alexine dans un même sérum. *Ann. Inst. Pasteur*, xv. 303.——(1903), Sur la mode de l'action des antitoxines sur les toxines. *Ann. Inst. Pasteur*, xvii. 162.

Bosse, B. (1903), Der Deyckesche Pepsin-Trypsin-Agar; ein Nährboden für Diphtheriebacillen. *Centralbl. f. Bakt.*, xxxii. 471.

Bouchard (1890), Sur les pretendues vaccinations par le sang. *Festschr. f. Virchow*, iii. 91.

Bowhill, T. (8. IV. 1899), Milk the vehicle of Contagion in an outbreak of Diphtheria. *The Veterinary Record*, No. 561.——(1899), *Manual of bacteriological technique and special bacteriology*, p. 163. Edinburgh.

Boycott, A. E. (7. IV. 1905), The seasonal prevalence of Hofmann's bacillus. *Journ. of Hygiene*, v. 223.

Brault (1880), Note sur les lésions du rein dans l'albuminurie diphthéritique. *Journ. de l'Anat. et de la Physiol.*, xvi. 673.

Braun (1901), Verbreitung der Diphtheritis und des Typhus des Menschen durch inficirte Kuhmilch. *Deutsche Landwirthschaftzeitung*, p. 278.

Braunschweig (1. XII. 1890), *Fortschr. d. med.* (Quoted by Stephenson, 1898.)

Bretonneau, P. (1826), *Traité de la diphthérite.* Des inflammations spéciales du tissu muqueux et en particulier de la diphthérite ou inflammation pelliculaire, connue sous le nom de croup, d'angine maligne, d'angine gangréneuse, etc. Paris.

Bretonneau, P. (I. 1855), Offener Brief an die Herren Blache und P. Guersant. *Arch. gener.* [translated in Behring, 1893, q.v.].

Brieger, L. (1895), Weitere Erfahrungen über Bakteriengifte. *Zeitschr. f. Hyg.*, xix. 101.

Brieger, L. and Boer (1895), Ueber Antitoxine und Toxine. *Zeitschr. f. Hyg.*, xxi. 259.——(3. XII. 1896), Ueber die Toxine der Diphtherie und des Tetanus. *Deutsche med. Wochenschr.*, xxii. 783.

Brieger and Ehrlich (1893), Beiträge zur Kenntnis der milch-immunisierten Tiere. *Zeitschr. f. Hygiene*, xiii. 336.

Brieger, L. and Fränkel, C. (1890), Untersuchungen über Bacteriengifte. *Berlin. klin. Wochenschr.*, 241.

Brieger, Kitasato and Wassermann (1892), Ueber Immunität und Giftfestigung. *Zeitschr. f. Hygiene*, xii. 137.

Brieger, L. and Wassermann, A. (1892), Beobachtungen über das Auftreten von Toxalbuminen bei Menschen. *Charité Annalen*, xvii.

Brodie, T. G. (1897), A preliminary report of some experiments upon the chemistry of the diphtheria antitoxin. *Journ. of Pathol. and Bacteriol.*, iv. 460.——(1899), The Physiological Action of Diphtheria Toxin. *Brit. Med. Journ.*, ii. 1282.

Bronstein, J. (12. II. 1900), Zur bacterioskopischen Diphtheriediagnose. *Berlin. klin. Wochenschr.*, xxxvii. 141.

Bronstein, J. and Grünblatt, G. N. (20. IX. 1902), Zur Frage über Differenzierung der Diphtherie- u. Pseudodiphtheriebacillen. *Centralbl. f. Bakt.*, xxxii. 425.

Brown, F. T. (VIII. 1890), Diphtheria of the meatus urinarius. *Journ. of Cut. a. Genito-urin. Dis.* (Ref. *Centralbl. f. Bakt.*, ix. 763.)

Brown, H. C. (XII. 1903), The presence and characteristics of bacilli morphologically resembling the Klebs-Löffler bacillus in vaccinia. *Journ. of Pathol. a. Bacteriol.*, ix. 154.

Brown, J. G. (1877), The Therapeutics of diphtheria; an experimental inquiry. *Journ. of Anat. and Physiol.*, xii. 1.

Brown, Lennox (1895), *Diphtheria and its associates.* London: Baillière, Tindall and Cox, 272 pp.

Brown, R. C. (1. III. 1902), Follicular Tonsillitis. *Medical Record*, lxi. 324.

Browne, L. (9. VII. 1894), False membrane of skin. *Lancet*, i. 1474. (Letter.)——(1894), Diphtheria: on doubts in diagnosis as determined by bacteriology. *Med. Press a. Circ.*, lviii. 608.——(1894–5), Three specimens illustrating the pseudo-diphtheria-bacillus otherwise the attenuated or non-toxic bacillus. *Trans. Path. Soc.*, xlvi. 301.

Brownlee, J. (1902), The antitoxin treatment of diphtheria in the Glasgow Fever Hospital during 6¼ years. *Glasgow Med. Journ.*, lvii.——(1905), Statistical studies in Immunity. *Journ. of Hyg.*, v. 514.

Bruck, C. (1904), Experimentelle Beiträge zur Theorie der Immunität. *Zeitschr. f. Hygiene*, xlvi. 176.——(1905), Ueber die Bindungsverhältnisse von Toxin und Antitoxin im homologen Organismus. Ein Beitrag zur Frage der antitoxischen Therapie mit homologem Tetanus-Antitoxin. *Ibid.* xlix. 282.

Brunner, C. (1893), Ueber Wunddiphtheritis. *Berlin. klin. Wochenschr.*, No. 22.

Bruno, J. (19. XII. 1898), Ueber Diphtherieagglutination und Serumdiagnostik. *Berlin. klin. Wochenschr.*, xxxv. 1127.

Buchner (1889), Ueber die nähere Natur der bakterientötenden Substanz im Blutserum. *Centralbl. f. Bakt.*, vi. 561.——(1889), Ueber bakterientötende Wirkung des zellenfreien Blutserums. *Ibid.* v. 817, also vi. 1.——(1890), Untersuchungen über die bakterienfeindlichen Wirkungen des Blutes und des Blutserums. *Arch. f. Hyg.*, x. 84.——(1893), Ueber Bakteriengifte und Gegengifte. *München. med. Wochenschr.*, xl. 449, 480.

Bugbee, M. L. (1904), Sequence of diphtheria cases. *American Med.*, viii. 318.

Buhl, L. (1867), Einiges über Diphtherie. *Zeitschr. f. Biol.*, iii. 341.

Bujwid, O. (30. III. 1897), Diphtheriebacillen in einem Harnsedimente. *Centralbl. f. Bakt.*, xxi. 394.——(1897), Ueber eine Methode der Conzentrierung der Diphtherie—u. anderen therapeutischen—Sera mittels Ausfrierung. *Ibid.* xxii. 287.

Bulloch, W. (1898), The duration of passive diphtheria immunity. *Journ. of Pathol. and Bacteriol.*, v. 274.——(1902), On the nature of hæmolysis and its relation to bacteriolysis. *Trans. Path. Soc.*, lii. 208.

Bullock und Schmorl (1894), Ueber Lymphdrüsenerkrankungen bei epidemischer Diphtherie. *Ziegler's Beiträge z. pathol. Anat.*, xvi. 247–255.

Bumm (1895), *Zeitschr. f. Geburtsh. u. Gynökol.*, xxxii. 126. (Ref. *Am. Journ. Med. Sci.*, cx. 110 [1905].)

Burfield, J. (1905), Two cases of diphtheritic infection of operation wounds. *St Barth. Hosp. Rep.*, xli. 233.

Burnett, F. M. (14. VII. 1900), Schools and diphtheria infectivity. *Brit. Med. Journ.*, ii. 90.

Burr, W. C. (1896), The nervous system and lesions of diphtheria. *Trans. Path. Soc.*, Philad., xvii. 284.

Büsing, E. (VIII. 1907), Beiträge zur Kenntnis der Diphtherie als Volksseuche, *Zeitschr. f. Hygiene*, lvii. 248.

Cache, A. (8. VII. 1901), De la culture du bacille de diphtérie croissant en fils ramifiés. *Centralbl. f. Bakt.*, xxix. 975.

Caddy, D. J. (4. III. 1893), Mammary diphtheria from suckling an infected infant. *Brit. Med. Journ.*, i. 457.

Cadet and Grassicourt (1895), Diagnostic et prophylaxie de la diphtérie. *La Semaine Méd.*, p. 280.

Cafiero, C. (1899), Sulla presenza del bacillo difterico nel sangue di alcuni animali de experimento. *Gazz. d. Osped.*, p. 1183. (Ref. *Baumgarten's Jahresber.*, xv. 257.)

Caiger, F. F. (1904), The diagnosis and management of doubtful cases of diphtheria. (*Issued by the Medical Officers of Schools Assoc.*) London: J. and A. Churchill, 16 pp., also *Lancet*, 1903, ii. 1771.

Calabrese, A. and Zagari, G. (1905), Ricerche sulla tossina ed antitossina difterica. *Giorn. internaz. d. Sci. med.*, No. 4. (Ref. *Baumgarten's Jahresber.*, xi. 215.)
——(1895 a), Ulteriori ricerche sulla tossina ed antitossina difterica. *Ibid.*, Nos. 19–21. (Ref. *Ibid.* xi. 216.)

Calmette, A. (1895), Contribution à l'étude des venins, des toxines et des sérums antitoxiques. *Ann. Inst. Pasteur*, ix. 225.

Calcar, R. P. van (1904), Ueber die Konstitution des Diphtheriegiftes; eine neue Methode zum Nachweise der Toxone. *Berlin. klin. Wochenschr.*, xli. 1028.

Cameron, A. G. R. (1905), Report *re* return cases of scarlet fever and diphtheria between July 1901 and July 1902. *Metropolitan Asylums Board, London.*

Campbell, R. R. (19. XI. 1904), Differential diagnosis between Pseudo-membranous angina of Syphilis and Diphtheritic angina. *Journ. Amer. Med. Assoc.*, xliii. (Ref. *Amer. Med.*, 1904, viii. 937.)

Camus, L. (1898), Résistance aux températures élevées des vaccins desséchées. *Compt. rend. Soc. de Biol.*, l. 235.

Cano-Brusso (1901), Ueber den Untergang des Tetanus Gifts in Darm. *Maly's Jahrb.*, xxxi. 914.

Canon (26. X. 1893), Bacteriologische Blutuntersuchungen bei Sepsis. *Deutsche med. Wochenschr.*, xix. 1039.

Capaldi, A. (28. XI. 1896), Zur Verwendung des Eidotters als Nährbodenzusatz. *Centralbl. f. Bakt.*, xx. 800.

Carnot and Fournier (20. II. 1901), Sur un cas d'angine à bacilles fusiformis. *La Semaine Méd.*, xxi. 60. (Report of Soc. de Biol. meeting, 9. II. 1901.)

Carpenter, A. (1883), Diphtheria: Its etiology and treatment. *Lancet*, ii. 448.

Carrière, G. (1899), Du sort de la toxine tétanique introduite dans le tube digestif. *Compt. rend. Soc. Biol.*, No. 8. (Ref. *Baumgarten's Jahresber.*, xv. 192.——(1899). Étude expérimentale sur le sort des toxines et des antitoxines dans le tube digestif des animaux. *Ann. Inst. Pasteur*, xiii. 435.

Carstairs, J. G. (IX. 1901), Unusual method of diphtheria infection. *Lancet*, ii. 765.

Carver, A. E. (1904), Diphtheria and Hofmann's bacillus. *Brit. Med. Journ.*, ii. 124.

Cary, C. and Lyon, I. P. (1901), Pseudo-membranous inflammation of the mucous membranes caused by pneumococcus. *Amer. Journ. Med. Sci.*, cxxii. 298.

Cassin, M. (1895). Fonctions protectrices actives de la muqueuse intestinale. *Sem. Méd.*, xv. 548.

Catrin (1890), La diphtérie chez les animaux domestiques. *Rev. d'Hyg.*, xii. 1051.

Cattani, G. (1890), Ueber die Reaktion der Gewebe auf specifische Reize. *Ziegler's Beitr. z. pathol. Anat. u. z. allgem. Pathol.*, vii. 171. (Ref. *Centralbl. f. Bakt.*, 1890, vii. 674.)

Cedrenus, G., *Compendium historiarum.* Pathologiae graecae, cxxii. 254. Numerus grandior, 522.

Cesaris-Demel, A. (16. XI. 1899), Ueber das verschiedene verhalten einiger Mikroorganismen in einem gefärbten Nährmittel. *Centralbl. f. Bakt.*, xxvi. 529.

Chaillou, A. and Martin, L. (VII. 1894), Étude clinique et bactériologique sur la diphtérie. *Ann. Inst. Pasteur*, viii. 419.

Chapin, C. V., *Report of Superintendent of Health. Providence, R.I.* Quoted by Massachussett's Committee, 1902.——(**XI. 1906**), The method of release of diphtheria patients in Providence. *Amer. Journ. of Public Hygiene*, xvi. 30.

Chappell, W. F. (1894), Vexed questions in the bacteriology of diphtheria. *Med. Record*, N.Y., xlv. 457.

Charrin (1898), Action des sucs digestifs sur les poisons microbiens. *Arch. de Phys.*, p. 67.

Charrin, A. and Gley, E. (1893), Recherches sur la transmission héréditaire de l'immunité. *Arch. de Physiol. norm. et pathol.*, p. 75.

Charrin, A. and Lefèvre (1897), Action de la pepsine sur la toxine diphtérique. *Compt. rend. Soc. Biol.*, iv. 830.

Charrin, A. and Levaditi (1899), Action du pancréas sur la toxine diphtérique. *Gaz. d. hôpitaux*, No. 40 ; also *Compt. rend. Soc. Biol.*, 215. (Ref. *Baumgarten's Jahresber.*, xv. 223.)

Charrin, A. and Thomas, A. (9. I. 1897), Lésion des cellules nerveuses chez un cobaye ayant présenté des accidents épileptiformes, à la suite d'infection de toxines diphtériques et d'une double amputation. *Compt. rend. Soc. de Biol.*, Series X., iv. 37.

Charsley, G. (1904), Diphtherial contacts. *Brit. Med. Journ.*, ii. 1750.

Chase (IV. 1900), *Journ. of Mass. Assoc. of Boards of Health.* (Quoted by *Denny.*)

Chatin and Lesieur (1900), *Rev. d'Hygiène*, xxii. 503.

Cioffi, E. (1897), Un altro caso di guarigione di endometrite difterica di origine puerperale, curato col siero di Behring. *Gazz. d. osped. e d. clin.*, No. 67. (Ref. *Centralbl. f. innere Med.*, xviii. 1007.)——(**1899**), La difterite uterovaginale nel puerperio; la vitalità dei bacilli di Klebs-Loeffler. *Riforma med.*, No. 268–271. (Ref. *Baumgarten's Jahresber.*, xv. 260.)

Class, W. J. (1898), Clinical significance of the different forms of the Klebs-Loeffler bacillus. *Journ. Amer. Med. Assoc.*, xxx. 1019.

Classen, A. (8. II. 1871), Beitrag zur Kenntniss der Diphtherie des Rachens. *Virchow's Arch.*, lii. 260.

Cobbett, L. (1896), Contribution to the study of the serum therapeutics of diphtheria. *Journ. of Pathol. and Bacteriol.*, iii. 327.——(**III. 1897**), Contribution à l'étude de la physiologie du bacille diphtérique. *Ann. Inst. Pasteur*, xi. 251–263.——(**5. II. 1898**), Alkalised serum as a culture medium for the bacteriological diagnosis of diphtheria. *Lancet*, i. 362.——(**10. III. 1898**), Alkalinisiertes Rinder und Pferdeserum als Hilfsmittel bei der Diphtheriediagnose. *Centralbl. f. Bakt.*, xxiii. 395.——(**17. IX. 1898**), Der Einfluss des Filtrierens auf das Diphtherie-Antitoxin. *Ibid.* xxiv. 386, 415.——(**3. XII. 1898**), The result of the treatment of diphtheria by Antitoxin in London compared with that in Paris and Berlin. *Lancet*, ii. 1457.——(**15. IV. 1899**), The resistance of rats to diphtheria toxin. *Brit. Med. Journ.*, i. 902.——(**5. VIII. 1898**), The origin of Antitoxin. Is it present in the blood of some normal animals? *Lancet*, ii. 332. (**16. IX. 1899**), Enthält das normale Pferdeserum Diphtherieantitoxin? *Centralbl. f. Bakt.*, xxvi. 548.——(**VI. 1900**), Has Antitoxin reduced the death-rate from Diphtheria in our large towns? *Edinburgh Med. Journ.*, N.S., vii. 251.——(**25. VIII. 1900**), Diphtheria in the Horse. *Lancet*, ii. 573.——(**30. XI. 1900**), Diphtheria beim Pferde. *Centralbl. f. Bakt.*, xxviii. 631.——(**1901**), Report on Measures taken to check the Outbreak of Diphtheria at Colchester in 1901. Colchester : 13 pp. 4°.——(**IV. 1901**), An Outbreak of Diphtheria checked by the Prophylactic Use of Antitoxin, and the Isolation of Infected persons. *Journ. of Hygiene*, i. 228.——(**IV. 1901**), The Result of 950 Bacteriological Examinations for Diphtheria Bacilli during an Outbreak of Diphtheria at Cambridge and Chesterton. *Ibid.* i. 235.——(**X. 1901**), Observations on the Recurrence of Diphtheria in Cambridge in the Spring of 1901. *Ibid.* i. 458. ——(**23. XI. 1901**), A note on Neisser's test for diphtheria bacilli. *Lancet*, ii.

1610.——**(1903)**, The nature of the so-called Pseudodiphtheria (Hofmann) Bacillus and its significance (if any) in the bacteriological examination for diphtheria. *Journ. of State Medicine*, xi. 608.——**(1904)**, The progress and prevention of Diphtheria. *Journ. Royal Sanitary Instit.*, xxv. 405.

Cobbett, L. and Kanthack, A. A. (6. VIII. 1898), Ueber das Schicksal des Diphtherietoxins im Tierorganismus. *Centralbl. f. Bakt.*, xxiv. 129.

Cobbett, L. and Melsome, W. S. (1898), Ueber den directen Einfluss der Entzündung auf die locale Widerstandsfähigkeit der Gewebe gegenüber der Infection. *Centralbl. f. allg. Path. u. path. Anat.*, ix. 827.

Cobbett, L. and Phillips, G. C. (XII. 1897), The Pseudo-Diphtheria Bacillus. *Journ. Pathol. and Bact.*, iv. 193.

Coggi, C. (1902), La diagnosi differenziale fra bacilli difterici e pseudodifterici con speciale riguardo al metodo di colorazione Piorkowski. *Giorn. d. R. soc. ital. d'igiene*, xxiv. 298. (Ref. *Baumgarten's Jahresb.*, xviii. 216.)

Cohn (1904), Erfahrungen über Serumbehandlung bei Diphtherie. *Mitteil a. d. Grenzgebiet der Med. u. Chir.*, xiii.

Cohnheim, J. (1882), *Allgemeine Pathologie*, i.

Coldstream, A. B. (1891), Case of diphtheria of the vulva. *Brit. Med. Journ.*, i. 1016.

Cole, W. C. (1894), A case of diphtheria in a child, transmitted from a chicken. *Arch. Pediat.*, N.Y., xi. 381.

Coleman, P. C. (1890), Diphtheria from cats. *Med. Record*, N.Y., xxxviii. 492.

Coles, A. C. (20. V. 1899), A modification of Neisser's diagnostic stain for the diphtheria bacillus. *Brit. Med. Journ.*, i. 1213.

Comba, C. (1894), Sulla alterazione del cuore nella difterite sperimentale. *Lo Sperimentale*, xlviii. 255–274.

Concetti, L. (1892), Sulla etiologia del Croup primitivo. *Arch. ital. di Pediatria*, x. (Ref. *Centralbl. f. Bakt.*, xii. 672.)——**(1892)**, Sulla difterite primativo cronica delle narici. *Arch. ital. di Laringol.*, xii. (Ref. *Centralbl. f. Bakt.*, xii. 673.)——**(1893)**, Ricerche sulla etiologia della difterite. *La Pediatria*, l, Nos. 3, 4, 5. (Ref. *Centralbl. f. Bakt.*, xiv. 629.)——**(1894)**, *Studi clinici e richerche sperimentali sulla diphtherite*. Roma.——**(1900)**, Rasche Methode zur bacteriologischen Diagnose der Diphtherie. *Wien. med. Wochenschr.* (Ref. *Centralbl. f. Bakt.*, xxviii. 712.)——**(1901)**, Ueber die actinomykotische Form des Loeffler'schen Bacillus in gewissen Zuständen saprophytischen Lebens. *Arch. f. Kinderheilk.*, xxxi. 227. (Ref. *Baumgarten's Jahresber.*, xvii. 168.)——**(1901)**, Forma actinomicotica del bacillo della difterite. *Ann. d'Ig. sper.*, xi. 404. (Ref. *Baumgarten's Jahresber.*, xvii. 174.)

Concetti, L. and Memmo, G. (1898), Sulla tossicità del bacillo di Loeffler, in rapporto alla sua morfologia. *Ann. d'Ig. sper.*, viii. 119. (Ref. *Centralbl. f. Bakt.*, xxv. 321.)

Conclin, W. L. (1902), Clinical versus bacteriological diagnosis and quarantine of diphtheria. *Buffalo Med. Journ.*, p. 660. (Ref. *Baumgarten's Jahresber.*, xviii. 207.)

Copeman (1892), in Stevenson and Murphy's *Treatise on Hygiene and Public Health*, i. 339.

Coppez, H. (1897), Des alterations cornéesnes dans la diphtérie de l'œil et du traitement local par le sérum. *Rev. gén. d'Ophthal.*, 197.——**X. (1899)**, Études sur la diphtérie oculaire. *Arch. d'Ophthalmol.* (Ref. *Centralbl. f. Bakt.*, xxviii. 148.)

Coppez, H. and Funck, M. (1895), Contribution à l'étude de l'étiologie et du diagnostic des ophthalmies pseudomembraneuses. *Journ. de Bruxelles*, p. 411. (Ref. *Baumgarten's Jahresber.*, xi. 253.)

Cornell, W. S. (27. X. 1906), The present treatment of diphtheria by the medical profession. *New York Med. Journ.*, LXXXIV. 835.——**(17. XI. 1906)**, The membrane and complications of diphtheria. A report of six hundred cases. *Ibid.* LXXXIV. 983

Cornil (1881), *Arch. de Physiol.*, p. 372.

Cornil, V. and Mégnin, P. (1885), Tuberculose et Diphtérie des Gallinacés. *Journ. de l'Anat.*, p. 268. (Ref. *Baumgarten's Jahresber.*, I. 74.)

Cornil and Ranvier (1869), *Manuel d'Histologie pathologique.*

Cornil and Babes (1890), *Les Bactéries.* Paris, II. éd.

Cortesius (1625), *Miscellaneorum medicinalium decades denae.* Messanae, Dec. IX. 696.

Coues, W. P. (4. XI. 1897), A case of diphtheria of the vulva. *Boston Med. and Surg. Journ.*, CXXXVII. 470.——**(12. V. 1898)**, Two additional cases of diphtheria of the vulva. *Ibid.* CXXXVIII. 445.——**(1903)**, A form of pressure anaemia of the uvula which may be mistaken for membrane. *Ibid.* CXLVIII. 178.

Councilman, W. T. (XI. 1893), The pathology and diagnosis of diphtheria. *Amer. Journ. Med. Sci.*, CVI. 540.——**(15. XII. 1896)**, An unusual action of the diphtheria bacillus. *Journ. of Boston Soc. of Med. Sci.*, I. 3.——**(1898)**, Acute interstitial nephritis. *Journ. Exper. Med.*, III. 393.

Councilman, W. T., Mallory, F. B. and Pearce, R. M. (1901), *A study of the bacteriology and pathology of two hundred and twenty fatal cases of diphtheria.* Monograph, 1901; also **(4. XII. 1900)**, *Journ. Boston Soc. Med. Sci.*, V. 137–319

Courmont, J. and Arloing, F. (12. I. 1901), Cytologie de la pleurésie diphtérique expérimentale du cobaye. *Compt. rend. Soc. de Biol.*, LIII. 40.

Courmont, J. and Doyon, M. (1895), De la marche de la température et de la vasodilatation dans l'intoxication diphtérique expérimentale. *Arch. de Physiol.*, XXVII. 252.

Courmont, J., Doyon and Paviot (1895), Lésions hépatiques engendrées chez le chien par la toxine diphtérique. *Compt. rend. Soc. Biol.*, 610. (Ref. *Baumgarten's Jahresber.*, XI. 209.)

Courtney, J. W. (1898), A case of pseudo-tabes following diphtheritic infection of the penis. *Atlantic Med. Weekly*, IX. 33.

Craig, C. F. (1896), Diphtheria: its bacteriology. *Trans. Amer. Microsc. Soc.*, XVIII. 271.——**(1904)**, A consideration of the efficacy of Antitoxin in the treatment of Diphtheria. *Albany Med. Annals*, XXV.

Craw, J. A. (1905), On the physical chemistry of the toxin-antitoxin reaction: with special reference to the neutralisation of lysin by antilysin. *Proc. Roy. Soc.* LXXVI. B. 179.

Craw, J. A. and Dean, G. (1907), On the Estimation of Free Diphtheria Toxin with relations existing between lethal doses, lethal times and loss in weight of the guinea-pig. *Journ. of Hygiene*, VII. 512.

Crocq (IX. 1895), [Diphtheritic lesions of the nervous system]. *Rev. mens. d. Malad. de l'Enfance.* (Rev. in *Amer. Journ. Med. Sci.*, CX. 628.)

Cronemeyer, G. (1895), *Beitrag zur pathologischen Anatomie den Difterie.* Inaug. Diss. Kiel., 13 pp.

Crookshank, E. M. (1896), *Text-book of Bacteriology*, 5th ed.

Crouch, H. C. (X. 1895), The detection of the diphtheria bacillus by its peculiar reaction towards certain stains. *New York Med. Journ.*, LXII. 430.——**(1896)**, The Microscopical Diagnosis of diphtheria by a new staining method. *Proc. Amer. Pub. Health Assoc.*, XXI. 19.

Crowley and Eurich (18. VI. 1904), *Brit. Med. Journ.*, I. 1431. (Report of Meeting, Bradford Medico-Chirurg. Soc.)

Cruveilhier, L. (1904), De la valeur thérapeutique des injections de sérum dans la diphtérie suivant les doses et la voie de pénétration. *Centralbl. f. Bakt.*, xxxv. 509.——**(1905)**, De la valeur thérapeutique de l'antitoxine dans le sérum antidiphtérique. *Ann. Inst. Pasteur*, xix. 249.

Cullen (1816), *First lines of the practice of physic*, i. 260. Philad. [Quotes Caldwell.]

Cuno, F. (23. X. 1902), Verlauf und Ursache einer Hospital-diphtherie Epidemie. *Deutsche med. Wochenschr.*, xxviii. 774.

Currie, J. R. (1907), On the supersensitation of persons suffering from diphtheria by repeated injections of horse serum. *Journ. of Hygiene*, vii. 35.——**(1907)**, Examples of the immediate and of the accelerated reaction following two injections of anti-diphtherial serum. *Ibid.* vii. 61.

Curtius (1904), Diphtherie und Kroup. (Cited by Fels.) *Wien. med. Presse*, xlv.

Czaplewski, E. (1898), Ueber einen aus einem Leprafalle gezüchteten Alkohol- und Saüre-festen Bacillus aus der Tuberkelbacillen Gruppe. *Centralbl. f. Bakt.*, xxiii. 97.——**(27. I. 1898)**, Die ätiologische Bedeutung des Loeffler'schen Bacillus. *Deutsche med. Wochenschr.*, xxiv. 55.

Czemetschka (1894), Ein Fall von Rhinitis diphtheritica bei einem Säuglinge. *Prag. med. Wochenschr.*, No. 38–39. (Ref. *Centralbl. f. Bakt.*, xvi. 974.)

Dahmer (1896), Untersuchungen über das Vorkommen von Streptokokken in Blut und inneren Organen von Diphtheriekranken. *Arb. a. d. pathol. anat. Inst. Tübingen*, Herausgegeben von Baumgarten, ii. 262. (Ref. *Centralbl. f. Bakt.*, xxii. 59.)

Daly, G. E. (1897), Diphtheria and Scarlet fever. *New York Med. Record*, li. 262. (Ref. *Baumgarten's Jahresber.*, xiii. 309.)

Danysz, J. (1902), Contribution à l'étude des propriétés et de la nature des mélanges des toxines avec leurs antitoxines. *Ann. Inst. Pasteur*, xvi. 331.

Darier, F. J. (1885), *De la bronchopneumonie dans la diphtérie.* Paris, 111 pp.

Darnier, J. (14. XI. 1885), Note sur les microbes de la bronchopneumonie diphtérique. *Compt. rend. Soc. Biol.*, Ser. VII., ii. 671.

D'Arsonval and Charrin (1896), Action des courants à haute fréquence sur les toxines bactériennes. *Compt. rend. Acad. d. Sci.*, 122.

Dávalos, J. N. (1894), La asociación del bacilo de Loeffler al Staphylococcus piogenes albus en un caso de impetigo de la cara. *Crónica méd.-quirurg. Habana.* No. 12–13. (Ref. *Centralbl. f. Bakt.*, xvii. 38.)

Davies, D. S. (16. IX. 1905), *Brit. Med. Journ.*, ii. 650.

Davis, L. D. (1898), Preliminary report on a bacillus resembling the diphtheria bacillus found in cultures in certain scarlet fever cases. *Proc. New York Path. Soc.*, p. 170.——**(1899)**, A bacillus resembling the diphtheria bacillus in all characteristics, but not producing diphtheria toxin. *New York Med. News*, lxxiv. 520.

Davison, J. T. R. (25. X. 1890), Notes on diphtheria in animals and in man; being a summary of an investigation carried out during an epidemic in Buenos Ayres. *Brit. Med. Journ.*, ii. 954.

Dean, G. (1900), Problems of Diphtheria Immunity. *Trans. Path. Soc., London*, v. 15.——**(1901)**, Experiments on Immunity in Relation to the Pancreas and its Ferments. *Trans. Path. Soc., London*, lii. 127.——**(I. 1905)**, Further observations on a leprosy-like disease of the rat. *Journ. of Hygiene*, v. 99.

Dean, G. and Todd, C. (IV. 1902), Experiments on the Relation of the Cow to Milk-Diphtheria. *Journ. of Hygiene*, ii. 194.

Debrie, E. (1892), Diphtérie humaine et diphtérie aviaire. Épidémies concomitantes. *Arch. de méd. et de Pharm. milit.*, 204; also Ref. *Centralbl. f. Bakt.*, xiii. 730.

Decius, H. (**1902**), Desinfektionsversuche mit chemisch reinem Wasserstoff-superoxyd. (*Inaug.-Diss.* Halle.) *Centralbl. f. Bakt.*, xxxiii. 239.

De Croly, O. (**1897**), Sur la disparition de la toxine diphtérique injectée dans le sang. *Arch. internat. de Pharmacodyn.*, iii. 61.——(**1898**), Étude de l'action des toxines et antitoxines sur la nutrition générale. *Ibid.* iv. 385.

Delacourt, A. (**1903**), [Croup due to the Streptococcus]. *Journ. Méd. de Brux.*, p. 196. (Rev. *Amer. Med.*, viii. 392.)

Delépine, S. (**1904**), Report upon an alleged effluvium nuisance attributed to the use of yeast in a tannery, and upon an outbreak of diphtheria. *Public Health Lab. Victoria Univ. of Manchester*, 32 pp. repr.

Demaria, E. B. (**1905**), Experimentelle Untersuchungen über antitoxische Wirkung der Tränen gegenüber dem Diphtherietoxin. *Klin. Monatsbl. f. Augenheilk.*, lxii. 246. (Ref. *Centralbl. f. Bakt.*, xxxvi. 396.)

Demartis, T. P. et de Vitray, A. B. (**1860**), *Nouveau traitement du croup et des angines couenneuses.* Paris.

Démétriadès (**1895**), Action de l'eau sur le bacille diphtérique. *Arch. de méd. exp.*, vii. (Ref. *Hyg. Rundsch.*, v. 1029.)

Denny, F. P. (**10. IV. 1900**), Report on the examination for diphtheria bacilli of cultures from four hundred and seventy-five well persons. *Journ. Boston Soc. Med. Sci.*, iv. 189.——(**15. XI. 1900**), Diphtheria bacilli in healthy throats and noses with reports of cases. *Boston Med. and Surg. Journ.*, cxliii. 515.——(**1902**), Notes on Morphology. *Amer. Pub. Health Assoc.*, xxviii. 465.——(**III. 1903**), Observations on the Morphology of *B. diphtheriae*, *B. pseudodiphtheriae*, and *B. xerosis*. *Journ. Med. Research*, ix. 117; also *Amer. Pub. Health Rep.*, xxxvii. 471–478.

Deschamps, E. (**1894**), Note sur un mode de propagation de la diphtérie. *Rev. d'hyg. et de police sanit.*, x. 241. (Ref. *Centralbl. f. Bakt.*, xvi. 791.)

D'Espine (**1886, 1888**), *Rev. méd. de la Suisse romande.* 1886, 584. 1888, No. 1, 49.

D'Espine, E. and de Marignac, E. (**1890**), Recherches expérimentales sur le bacille diphtérique. *Rev. méd. de la Suisse rom.*, x. Nos. 1 and 2, 34. (Ref. *Baumgarten's Jahresber.*, vi. 341.)

Dessaux (**1902**), Sérothérapie et Diphtérie. *La Normandie méd.*, xvii.

Detot, E. (**1903**), Les Erythèmes provoqués par le sérum antidiphtérique. *Arch. gén. de Méd.*, lxxx. 2588.

Deucher, P. (**1895**), Zur klinischen Diagnose der Diphtherie. *Korrespondenzbl. d. Schweizer Aerzte*, No. 16. (Ref. *Centralbl. f. Bakt.*, xviii. 553.)

Deycke, G. (**28. VI. 1898**), Ueber die Absterbebedingungen pathogener Keime auf gewissen Anstrichfarben. *Centralbl. f. Bakt.*, xxiii. 1033.

Deyl, J. (**1893**), Ueber die Aetiologie des Chalazion. (Ref. *Centralbl. f. Bakt.*, xiv. 404.)

Dieudonné, A. (**1897**), Ueber Diphtheriegift neutralisierende Wirkungen der Serumglobuline. *Arb. a. d. kaiserl. Gesundheitsamte*, xiii. 293.——(**1897**), Ergebnisse der Sammelforschung über das Diphtherieheilserum für die Zeit vom April 1895 bis März 1896. *Ibid.* xiii. 254.——(**1900**), *Schutzimpfung u. Serumtherapie.* J. A. Barth, Leipzig, 1900.

Dieulafoy (**1895**), De l'angine diphtérique à forme herpétique. *Bull. de l'Acad. de Méd.*, lix. (Ref. *Baumgarten's Jahresber.*, xi. 265.)

Dolschenkow (**1873**), *Centralbl. f. d. med. Wiss.*, Nos. 42–43.

Donkin, H. B. (**1900**), The diagnosis of diphtheria. *Brit. Med. Journ.*, ii. 1303.

Dönitz, W. (**1899**), Ueber die Grenzen der Wirksamkeit des Diphtherie-Heilserums. *Arch. internat. de Pharmacodyn.*, v. 425. (Ref. *Baumgarten's Jahresber.*, xv. 228.)

Dotsch, A. (1900), Anatomische und bakteriologische Untersuchungen über infantile Xerosis und Keratomalacis, sowie Bemerkungen über Verhornung des Bindehaut- und Hornhaut-epithels. *Graefe's Archiv*, xlix. (Ref. *Centralbl. f. Bakt.*, xxvii. 691.)

Douglas, J. S. C. (1904), The spread and control of diphtheria epidemics. *Med. Times and Hosp. Gaz.*, xxxii. 17.

Downes, A. (12. IV. 1879), Note on diphtheria diffusion. *Lancet*, i. 515.

Dowson, W. (1893), *Report of Med. Officer of Health*, Bristol.

Draeer, A. (5. VII. 1894), Ueber die Disinfectionskraft der Sozojodolsäure und verschiedener ihrer Salze gegenüber dem Löfflerschen Diphtherie-bacillus. *Deutsche med. Wochenschr.*, xx. 567.——(30. IV. 1896), Die bacteriologische und klinische Diagnose "Diphtherie." *Ibid.* xxii. 279.

Dreyer, G. (1901), Ueber die Grenzen der Wirkung des Diphtherieheilserums gegenüber den Toxonen des Diphtheriegiftes. *Zeitschr. f. Hyg.*, xxxvii. 268.

Dreyer, G. and Madsen, T. (1901), Ueber Immunisirung mit den Toxonen des Diphtheriegiftes. *Zeitschr. f. Hyg.*, xxxvii. 250.——(1902), Studies on Diphtheria Toxin. *Contrib. Univ. Lab. for Med. Bacteriol., to celebrate the Inauguration of the Serum Inst.*, Paper No. 5, 1 Chart, pp. 3–6. Copenhagen.

Dubief and Bruhl (21. II. 1891), Note sur une altération des cellules hépatique dans la diphtérie expérimentale. *Compt. rend. Soc. de Biol.*, Ser. IX., iii. 135.

Duchamp, L. G. (1858), *Des parasites de la diphtérie.* Thèse, Paris.

Duclaux, F. (1882), *Ferments et maladies.* Paris.

Dumas, S. (1898), *Contribution à l'étude de la diphtérie toxique pure.* Paris.

Dungern, E. von (1896), Die Bedeutung der Mischinfektion bei Diphtherie. *Ziegler's Beiträge*, xxi. 1. (Ref. *Centralbl. f. Bakt.*, xxi. 346.)——(1896), Steigerung der Giftproduktion des Diphtheriebacillus. *Centralbl. f. Bakt.*, xix. 137.——(1897), Die Bedeutung der Mischinfektion bei Diphtherie. *Ziegler's Beiträge*, xxi. 104.——(1900), Beiträge zur Immunitätslehre. *München. med. Wochenschr.*, No. 20.——(1902), *Die Antikörper. Resultate früherer Forschungen und neue Versuche.* Jena, G. Fischer.——(1904), Beitrag zur Kenntnis der Bindungsverhältnisse bei der Vereinigung von Diphtheriegift und Antiserum. *Deutsche med. Wochenschr.*, xxx. 275.

Dunham, J. D. (1899), Diphtheria; etiology; diagnosis; prophylaxis. *New York Med. News*, lxxv 417. (Ref. *Baumgarten's Jahresber.*, xv. 206.)

Durante, D. (1902), Sulla batteriologia del Noma. *La Pediatria*, x. (Ref. *Centralbl. f. Bakt.*, xxxiii. 398.)

Dzierzgowski, S. J. (1897), Zur Frage, Ueber das Verhalten des Diphtherieheilserums bei der Filtration durch das Chamberland'sche Filter. *Centralbl. f. Bakt.*, xxi. 33.——Ueber den Gehalt an Antitoxin in den Körperflüssigkeiten und den einzelnen Organen der gegen Diphtherie immunisirten Pferde. *Arch. f. exper. Pathol.*, xxxviii. 186.——(1899), De l'immunisation des animaux contre la diphtérie et de la préparation du sérum antidiphtérique. *Arch. d. Sci. biol. de St Petersbourg*, ix. 293.——(1899), Die Beziehung der Verdauungsfermente, zum Antidiphtherieserum. *Arch. de Sci. Biol.*, vii. 337.——(1901), Ein Beitrag zur Frage der Vererbung der künstlichen Diphtherieimmunität. *Centralbl. f. allg. Pathol. u. pathol. Anat.*, xii. 715.

Dzierzgowski, S. and de Rekowski, L. (1895), Recherches sur la transformation des milieux nutritifs par les bacilles de la diphtérie et sur la composition chemique de ces microbes. *Arch. d. sci. biol.*, publ. par l'Inst. imp. de méd. expér. à St Pétersbourg, i. 167. (Ref. *Centralbl. f. Bakt.*, xvii. 466.)

Eastes, G. L. (24. I. 1903), *Lancet*, i. 237. (Report Meeting Pathol. Soc. London.)

Eberlein (1894), Geflügeldiphtherie bei Rebhühnern. *Monatsh. f. prakt. Tierheilk.*, v. 433. (Ref. *Centralbl. f. Bakt.*, xvii. 137.)

Eberth, C. J. (1872), *Cor.-Bl. f. d. schweiz. Aerzte*; also *Ueber bacter. Mycosen.* Leipzig, 1872.——(1873), Die diphtherischen Processe. *Centralbl. f. d. med. Wissensch.*, xi. 113.——(1873), Zur Kenntniss der Wunddiphtherie. *Ibid.* xi. 291.

Editorial (19. IX. 1900), Diphtheria in the Horse. *Journ. of Comp. Path. and Therap.*, xiii. 246.

Edlefsen (1883), *Verhandl. d. Congr. f. inn. Med.*, Wiesbaden.

Eeman (1896), De la diphtérie nasale. *Bull. Soc. belge d'otol. et de laryngol.*, i. 9.

Eeman and Bayer (1896), Rhinite pseudo-membraneuse et diphtérie nasale. *Ibid.* i. 42.

Ehrhardt, P. and Favre, A. (1890), Recherches cliniques et bactériologiques sur une épidémie bénigne et diphtérie puerpérale à la Maternité de Paris. *N. Arch. d'Obst. et de gynéc.*, v. 416.

Ehrlich, P. (1885), *Die Sauerstoffbedürfnisse des Organismus.*——(1892), Ueber Immunität durch Vererbung und Säugung. *Zeitschr. f. Hygiene*, xii. 183.——(1896), *Die Werthbemessung des Diphtherieserums.* Jena.——(1897), *Klinisches Jahrbuch.*——(1898), Ueber die Constitution des Diphtheriegiftes. *Deutsche med. Wochenschr.*, xxiv. 597.——(1900), Croonian Lectures. On immunity with special reference to cell life. *Proc. Roy. Soc.*, lxvi. 424.——(1901), Ueber Toxine und Antitoxine. *Ther. der Gegenwart*, Berl., n. F., iii. 193.——(1903), Toxin und Antitoxin. Entgegnung auf den neuesten Angriff Gruber's. *München. med. Wochenschr.*, Nos. 33–34. Republ. in Ehrlich, 1904, pp. 727–776.——(1903), Ueber die Giftcomponenten des Diphtherie-Toxins. *Berlin. klin. Wochenschr.*, 35–37. Republished in Ehrlich, 1904 and 1906, pp. 680–726.——(1904), *Gesammelte Arbeiten zur Immunitätsforschung.* Berlin: Hirschwald, 776 pp., 12 figs.——(1904), Ueber die Beziehungen von chemischer Constitution, Vertheilung und pharmakologische Wirkung. *v. Leyden's Festschr.*, 573.——(1904), Toxin and antitoxin. *München. med. Wochenschr.*, li. 1428, 1465.——(1904), Ueber die Giftcomponenten des Diphtherie-Toxines. *Berlin. med. Wochenschr.*, xli. 680.——(1904), Vorläufige Bemerkungen zur Mitteilung von Arrhenius. *Berlin. klin. Wochenschr.*, xli. 222.——(1906), *Collected Studies on Immunity*, translated by Dr C. Bolduan. New York: John Wiley and Sons.

Ehrlich, P. and Hübener (1894), Ueber die Vererbung der Immunität bei Tetanus. *Zeitschr. f. Hygiene*, xviii. 51.

Ehrlich, P. and Kossel, H. (1894), Ueber die Anwendung des Diphtherieantitoxins. *Zeitschr. f. Hygiene*, xvii. 486.

Ehrlich, P., Kossel, H. and Wassermann, A. (1894), Ueber Gewinnung und Verwendung des Diphtherieheilserums. *Deutsche med. Wochenschr.*, xx. 353.

Ehrlich, P. and Wassermann, A. (1894), Ueber die Gewinnung der Diphtherie-antitoxin aus Blutserum und Milch immunisierter Tiere. *Zeitschr. f. Hygiene*, xviii. 239.

Eigenbrodt (1894), Ueber den Einfluss der Familiendisposition auf die Verbreitung der Diphtherie. *Deutsche Vierteljahrsschr. f. öffentl. Gesundheitspfl.*, xxv. 517. (Ref. *Centralbl. f. Bakt.*, xv. 759.)

Eisenberg, P. (1903), Ueber die Bindungsverhältnisse zwischen Toxin und Antitoxin. *Centralbl. f. Bakt.*, xxxiv. 259.

Ellerman, V. (1905), Ueber die Kultur der fusiformen Bacillen. *Centralbl. f. Bakt.*, xxxvii. 729.

Elsner, S. L. (1898), Report of a case of puerperal diphtheria involving vagina and endometrium. *Buffalo Med. Journ.*, xxxvii. 842. (Ref. *Baumgarten's Jahresber.*, xiv. 309.)

Emmerich, R. (1884), Ueber die Ursachen der Diphtherie des Menschen und der Tauben. *Deutsche med. Wochenschr.*, x. 614.

Engel, C. S. (1896), De l'examen du sang au point de vue du prognostic de la diphtérie. *La Semaine Méd.*, xvi. 277. (Notice of Meeting Soc. Intern. Méd. Berlin, 2. VII. 1896.)——**(18. II. 1897)**, Hämatologischer Beitrag zur Prognose der Diphtherie. *Deutsche med. Wochenschr.*, xxiii. 118, 137.

Enriquez and Hallion (6. I. 1894), *Lancet*, i. 65 (review).——**(1894)**, Myélite expérimentale par toxine diphtérique. *Compt. rendu Soc. Biol.*, Ser. X., i. 312. ——**(1895)**, Sur les effets physiologiques de la toxine diphtérique. *Arch. de Physiol.*, xxvii. 515.

Epstein, A. A. (30. X. 1906), A new simple method for staining the polar bodies of diphtheria bacilli. *Journ. of Infect. Diseases*, iii. 770.

Erbrich, F. (1901), Neuere Untersuchungsmethoden der Diphtheriebacillen. *Gazeta Lekarska.* (Ref. *Centralbl. f. Bakt.*, xxxii. 83.)

Ergebnisse einer Umfrage bei Aerzten des Deutschen-Reiches betreffend die Erfolge der Schutzimpfungen mit Diphtherieheilserum (1903). *Med. Statistische Mitteil. a. d. k. Gesundheits.* Berlin, viii.

Erikson, E. A. (1903), Fall von Vulvovaginitis durch Diphtheriebacillen verursacht. *Hygiea*, i. 651. (Ref. *Baumgarten's Jahresber.*, xix. 224.)

Ernst, P. (1888), Ueber den Bacillus xerosis und seine Sporenbildung. *Zeitschr. f. Hyg.*, iv. 25. (See also *ibid.*, 1889, v. 428.)

Eröss (1904), Uber die Mortalität der Diphtherie und des Croups in den grösseren Städten Ungarns, etc. *Jahrb. f. Kinderheilk.*, lx.

Escherich, T. (2. I. 1890), Zur Aetiologie der Diphtherie. *Centralbl. f. Bakt.*, vii. 8. ——**(1890)**, *Festschr. z. 70. Geburtstag von Henoch Berlin.*——**(1893a)**, Zur Frage des Pseudodiphtheriebacillus und der diagnostischen Bedeutung des Loefflerschen Bacillus. *Berlin. klin. Wochenschr.*, No. 21–23. (Ref. *Baumgarten's Jahresber.*, ix. 192.)——**(1893b)**, Die Aetiologie und die Pathogenese der Diphtherie. *Wien. med. Wochenschr.*, No. 47–50.——**(1894)**, *Aetiologie und Pathogenese der epidemischen Diphtherie.* i. *Der Diphtherie bacillus.* 294 pp. Wien: Alf. Hölder.——**(1895)**, *Diphtherie, Croup und Serumtherapie.* Wien. ——**(1897)**, Versuche zur Immunisierung gegen Diphtherie auf dem Wege des Verdauungstractus. *Wien. klin. Wochenschr.*, x. 799.

Escherich and Klemensiewicz (1893), Ueber einen Schutzkörper im Blut der von Diphtherie geheilten Menschen. *Centralbl. f. Bakt.*, xiii. 153.

Eschweiler (1900), Spätdiphtherie im Nasenrachenraum. *München. med. Wochenschr.*, No. 17, p. 568.

Esmarch, T. von (3. I. 1895), Die Durchführung der bacteriologischen Diagnose by Diphtherie. *Deutsche med. Wochenschr.*, xxi. 7.

Eve, F. C. (1904), A small diphtheritic epidemic. *Brit. Med. Journ.*, i. 1192.

Everett (1881), *Med. and Surg. Reporter*, Philadelphia, xiv. 197–229.

Ewart, W. (IX. 1900), Can the period of infectiveness in diphtheria be shortened and its tendency to spread diminished? *Edin. Med. Journ.*

Ewing (1901), *Clinical pathology of the blood.* New York: Lea Brothers.

Eyre, J. W. H. (1896), On the Xerosis Bacillus. *Trans. Path. Soc.*, xlvii. 334.——**(1897)**, On the Xerosis Bacillus. *Journ. Path. and Bact.*, iv. 54.——**(2. IX. 1899)**, The Bacillus diphtheriae in Milk. *Brit. Med. Journ.*, ii. 586.——**(18. VIII. 1900)**, On the presence of members of the diphtheria group of bacilli other than Klebs-Loeffler bacilli in milk. *Ibid.* ii. 426.——**(XII. 1904)**, The bacterial content of milk at the point of origin and distribution. *Journ.*

of State Med., xii. 728.——(**9. XII. 1905**), General paralysis of the insane and diphtheroid infection. *Brit. Med. Journ.*, ii. 1557 (Letter).

Eyre, J. W. H. and Flashman, J. F. (28. X. 1905), Diphtheroid organisms in the throats of the insane. *Brit. Med. Journ.*, ii. 1104.

Faber, K. (1890), Die Pathogenese der Tetanus. *Berlin. klin. Wochenschr.*, xxvii. 717.——(**1904**), Serumbehandlung bei Diphtherie. *Jahrb. f. Kinderheilk.*, lix.

Faquet, E. (1896), *Recherches sur la diphtérie aviaire et ses rapports avec la diphtérie de l'homme.* Thèse, Bordeaux. (Ref. *Baumgarten's Jahresber.*, ii. 301.)

Falières (1902), Des granulations polaires du bacille diphtérique. [Dissertation.] (Quoted by Blumenthal and Lipskerow.)

Favre, A. (1890), Recherches cliniques et bactériologiques sur une épidémie de diphtérie puerpérale à la Maternité de Paris. *Nouv. Arch. de Gynécol.* (Ref. *Baumgarten's Jahresber.*, vi. 350.)

Federici, F. (1893), Sulla presenza del bacillo di Loeffler nel sangue dei difterici. *Arch. ital. di clin. med.* (Ref. *Baumgarten's Jahresber.*, ix. 196.)

Feer, E. (1893), Echte Diphtherie ohne Membranbildung unter dem Bilde der einfachen katarrhalischen Angina. *Korrespdzbl. f. Schweiz. Aerzte*, xxiii. (Ref. *Centralbl. f. Bakt.*, xiv. 366.)——(**1894**), *Aetiologische und klinische Beiträge zur Diphtherie.* Basel and Leipzig: Carl Sallmann.——(**1895**), Zur Diagnose der Diphtherie. *Correspdzbl. f. Schweiz. Aerzte.* (Ref. *Baumgarten's Jahresber.*, xi. 188.)

Feinberg (1898), Ueber das Verhalten des Klebs-Loeffler'schen Diphtheriebacillus in der Milch, nebst einigen Bemerkungen zur Sterilisation derselben. *Zeitschr. f. klin. Med.*, xxxiii. 432. (Ref. *Baumgarten's Jahresber.*, xiv. 254.)——(**31. III. 1900**), Ueber den Bau der Bakterien. *Centralbl. f. Bakt.*, xxviii. 415.

Felt, C. L. (14. II. 1903), Post-diphtheritic paralysis affecting the ear and throat. *Med. News*, lxxxii. 3.

Felix, J. (1870), Beiträge zur Kenntniss der epidemischen Diphtheritis. *Wien. med. Wochenschr.*, xx. 844.

Fels (1904), Diphtherie und Kroup mit und ohne Serum behandelt. *Wien. med. Presse*, xlv.

Fennyvessy, B. (1896), Ueber die Wirkung des Diphtherietoxins und Antitoxins auf das Froschherz. *Jahresb. f. Kinderheilk.*, xliii. 216.

Fermi, C. (11. II. 1898), Die mineral- und organischen Säuren, die Alkali, die Alkaloide, das Jodkali, und das arsensaure Kali zur Differenzierung der Mikroorganismen. *Centralbl. f. Bakt.*, xxiii. 208.

Ferré, G. (1896), De quelques résultats fournis par la pratique régulière du diagnostic bactériologique de la diphtérie. *Assoc. franc. pour l'avance. d. sc.*, xxiv. 815.——(**1897**), La diphtérie à Bordeaux. *Arch. clin. de Bordeaux*, p. 195. (Ref. *Baumgarten's Jahresber.*, xiii. 304.)——(**1898**), Diphtérie humaine et diphtérie aviaire. *La Semaine Méd.*, xviii. 163. (Report of 9th International Congress of Hygiene and Demography at Madrid, April, 1898.)

Fibiger, J. (1895), *Bakteriologiske Studier over Diphteri*, Copenhagen, p. 163. (Ref. *Centralbl. f. Bakt.*, xviii. 450.)——(**1897**), Ueber Bekämpfung von Diphtherieepidemien durch Isolirung der Individuen mit Diphtheriebacillen in Schlunde. *Berlin. klin. Wochenschr.*, xxxiv. 753. (See also *Centralbl. f. Bakt.*, xxiii. 564.)

Ficker, M. (15. XI. 1902), Eine neue Methode der Färbung von Bakterienkörnchen. *Hyg. Rundsch.*, xii. 1131.

Field, C. W. and Teague, O. (1907), The electrical charge of toxin and antitoxin. *Journ. of Exper. Med.*, ix. 86.

Fischer, E. (1830), De anginae membranaceae origine et antiquitate, p. 37, Berol. [Quotes Harris und Struve.]

Fischl, J. (1883), Zur Kenntniss der Nierenaffection bei der Diphtherie. *Zeitschr. f. klin. Med.*, vii. 427-458.

Fischl, R. and von Wunschheim (1895), Ueber Schutzkörper im Blute des Neugeborenen ; das Verhalten des Blutserums des Neugeborenen gegen Diphtheriebacillen und gegen Diphtherie. *Prag. Zeitschr. f. Heilk.*, xvi. 429.

Flexner, S. (1892), The Histological Lesions produced by the Toxalbumin of Diphtheria. *Johns Hopkins Hosp. Bull.*, 1892, iii.——(**IV. 1893**), Diphtheria with Bronchopneumonia. *Ibid.* iv. 32.——(**III. 1894**), Fatty degeneration of the heart muscle. *Ibid.* v. 26.——(**III. 1895**), The Bacteriology and Pathology of Diphtheria. *Ibid.* v. p. 39 ; also *Amer. Journ. Med. Sci.*, cix. 240.——(**1897**), The pathology of toxalbumin intoxication. *Johns Hopkins Hospital Reports*, vi. 259-409.

Flexner, S. and Anderson, H. B. (**IV. 1898**), The results of the intratracheal inoculation of the Bacillus diphtheriae in rabbits. *Johns Hopkins Hosp. Bull.*, ix. 72-79.

Flexner, S. and Pease, H. D. (**II. 1895**), Primary diphtheria of the lips and gums. *Johns Hopkins Hosp. Bull.*, No. 47, p. 22.

Fodor, J. von (**1886**), Neuere Versuche mit Injection von Bakterien in die Venen. *Deutsche med. Wochenschr.*, xii. 617.——(**1887**), Die Fähigkeit des Blutes Bacterien zu vernichten. *Ibid.* xiii. 745.

Fontecha, de (**1611**), Disputationes medicae super ea quae Hippocrates, Galenus, Avicenas necnon et alii Graeci, Arabi et Latini de Anginarum naturis, speciebus, causis etc., curiationibus scripsere diversis in locis, et circa affectionem hisce temporibus vocatam Garrotillo. Compluti.

Forbes (**1895**), *Wien. med. Presse.*

Forbes, D. (**V. 1903**), Preliminary note as to the frequency and importance of the presence of diphtheria bacilli in the ear discharges of Scarlet fever patients. *Journ. of Path. and Bacteriol.*, viii. 448.——(**1904**), *Appendix of Annual Report of M.O.H. of Manchester.*

Forest (**1904**), Diphtherie und Kroup, mit und ohne Serum behandelt. *Wien. med. Presse*, xlv.——(**1905**), Diphtherie und Kroup bei Neugeborenen. *Arch. f. Kinderheilk.*, xlii.

Forestus, P. (**1591**), Observationum et curationum medicinalium de febribus publice grassantibus etc. Liber Sextus, p. 10, Lugd. Bat. ex off. Plantiniana.

Forssmann, J. (**1905**), Studien über die Antitoxinbildung bei aktiver Immunisierung gegen Botulismus. *Centralbl. f. Bakt.*, xxxviii. 463.

Forssmann and Lundstrom (**1902**), Sur la marche de la curve d'antitoxine dans l'immunisation active contre le Botulisme. *Ann. Inst. Pasteur*, xvi. 294.

Förster (**1900**), Versuche über Wäschedesinfektion. *Hyg. Rundsch.*, x. 513.

Fothergill, J. (**1748**), *An account of the sore throat attended with ulcers ; a disease which of late years appeared in this city and the parts adjacent.* London.

Foulerton (**1902**), Pneumococcic gastritis, with some remarks on the infection of mucous membranes by Diplococcus pneumoniae. *Trans. Path. Soc.*, liii. 293.

Foulerton, A. G. R. and Bonney, V. (**1903**), A "diphtheroid" bacillus isolated from two cases of puerperal fever. *Trans. Path. Soc.*, liv. 139.——(**8. IV. 1905**), An investigation into the causation of puerperal infection. *Lancet*, i. 915, 992.

Foulerton, A. G. R. and Williams, A. L. (**23. X. 1897**), On the conveyance of diphtheritic infection by apparently healthy individuals. *Lancet*, ii. 1038.

Fraenkel and Franke (**1887**), *Archiv. f. Augenheilk.*, vii. [Quoted by Stephenson, 1898.]

Fraenkel, C. (1890), Immunisierungsversuche bei Diphtherie. *Berl. klin. Wochenschr.*, iii. 1133.——(1893), Ueber das Vorkommen der Loeffler'schen Diphtheriebacillen. *Ibid.* No. 11. (Ref. *Hyg. Rundsch.*, iii. 541.)——(1894), Beiträge zur Kenntniss des Bakterienwachstums auf eiweissfreien Nährlösungen. *Ibid.* iv. 769.——(14. **III.** 1895), Die ätiologische Bedeutung des Loeffler'schen Bacillus. *Deutsche med. Wochenschr.*, xxi. 172.——(15. **IV.** 1895), Eine morphologische Eigenthümlichkeit des Diphtheriebacillus. *Hyg. Rundsch.*, v. 349.——(**X.** 1896), Zur Unterscheidung des echten und des falschen Diphtheriebacillus. *Ibid.* vi. 977; also (1897) *Berlin. klin. Wochenschr.*, No. 50.——(1896), Die Bekämpfung der Diphtherie. *Ibid.* No. 40–41. (Ref. *Baumgarten's Jahresber.*, xii. 297.)

Fraenkel, E. (3. **I.** 1896), Beiträge zur Pathologie und Aetiologie der Nasennebenhöhlen-Erkrankungen. *Virchow's Arch.*, cxliii. 42.

Francotte, X. (1883), *La diphtérie considérée principalement au point de vue de ses causes, de sa nature, et de son traitement.* Bruxelles.

Franke (1893), *Arch. f. Ophthal.*, xxxix. 529. [Quoted by Bergey, 1898.]——(1898), Xerose-, Diphtherie-, und Pseudodiphtheriebacillen. *München. med. Wochenschr.*, No. 16.——(20. **X.** 1898), Die sogenannten Xerosebacillen und die Pseudodiphtheriebacillen des Auges. *Deutsche med. Wochenschr.*, xxiv. 675.

Franklin, G. H. (1893), An epidemic of diphtheria in Hightstown, New Jersey, in July 1893, supposed to have been caused by infected milk. *Internat. Med. Mag.*, Philad., ii. 816.

Fraser, T. (1896), Immunisation against serpent's venom. *Nature*, 569.

Freeman, W. J. (1896), Diphtheria of the naso-pharynx. *Med. Rec.*, N.Y., i. 618. (Ref. *Baumgarten's Jahresber.*, xii. 293.)

Freund and Joachim (1902), Zur Kenntniss der Serumglobuline. *Zeitschr. f. physiol. Chem.*, xxxvi. 407.

Freund, E. and Sternberg, C. (1899), Ueber Darstellung des Heilkörpers aus dem Diphtherieheilserum. *Zeitschr. f. Hygiene*, xxxi. 429.

Freymouth and Petruschky (14. **IV.** 1898), Ein Fall von Vulvitis gangraenosa (Noma genitalium) mit Diphtheriebacillenbefund. Behandlung mit Heilserum. Heilung. *Deutsche med. Wochenschr.*, xxiv. 232.——(22. **IX.** 1898), Zweiter Fall von Diphtherie—Noma,—Noma facei—Behandlung mit Heilserum; Herstellung. *Ibid.* xxiv. 600.

Friedberger and Fröhner (1904), *Veterinary Pathology*, i. 221. 4th edition. Translated by Hayes, M. H.

Frosch, P. (1893), Die Verbreitung des Diphtheriebacillus im Körper des Menschen. *Zeitschr. f. Hyg.*, xiii. 49–53.

Fuld and Spiro (1900), Ueber die labende und labhemmende Wirkung des Blutes. *Zeitschr. f. physiol. Chem.*, xxxi. 132.

Funck (1894), Experimentelle Studien über die Frage der Mischinfection bei Diphtherie. *Zeitschr. f. Hyg.*, xvii. 465.

Funke, J. (9. **XI.** 1901), The bacteriology of otitis media; a summary of recorded observations and a laboratory study of 76 cases. *American Med.* (Reprinted in *Publicat. from Labs. of Jefferson Med. Coll. Hosp.*, Vol. i., 1904.)

Fürbringer, P. (1883), Zur Klinik und pathologischen Anatomie der diphtherischen Nephritis. *Virch. Arch.*, xci. 385–410.

Furgus, F. (11. **III.** 1905), Some practical aspects of conjunctival bacteriology. *Brit. Med. Journ.*, i. 522.

Fürst (**III.** 1895), Die klinische und bakterielle Frühdiagnose bei diphtherieverdächtigen Anginen. *Berliner klinik. Sammlung klin. Vorträge*, Hft. 81. (Ref. *Centralbl. f. Bakt.*, xvii. 578.)

Fussel, M. H. (16. **XI.** 1901), The value of throat cultures in diphtheria. *Journ. Amer. Med. Assoc.*

Gabritschewsky, G. (X. 1894), Du rôle des leucocytes dans l'infection diphtérique. *Ann. Inst. Pasteur*, VIII. 673.——**(1901)**, Zur Prophylaxe der Diphtherie. *Zeitschr. f. Hyg.*, XXXVI. 45.——**(1905)**, Die Immunisation und die Verdauungstrakten. *Centralbl. f. Bakt.*, XLVI. 466.

Gallenga (1890), *Ann. di Neuropath.*, f. 4–5. [Quoted by Stephenson, 1898.]

Gallez, L. (1895), Diphtérie animale et diphtérie humaine. *Presse méd. belg.*, No. 22, 23. (Ref. *Baumgarten's Jahresber.*, XI. 189.)——**(1896 a)**, Recherches expérimentales sur l'origine aviaire de la diphtérie. *La Semaine Méd.*, XVI. 136. (Meeting of Acad. de Méd. de Belgique, 28. III. 1896.)——**(1896 b)**, Recherches expérimentales sur l'origine aviaire de la diphtérie humaine. *Bull. de l'Acad. roy. de Méd. de Belgique*, p. 218. (Ref. *Baumgarten's Jahresber.*, XII. 301.)

Galli-Valerio, B. (24. XI. 1897), L'état actuel de la question sur l'identité de la diphtérie de l'homme et des oiseaux. *Centralbl. f. Bakt.*, XXII. 500.——**(1904)**, Études Bactériologiques : Corynebacterium vaccinae.—Bacterium diphtheriae avium.—Bacterium candidus. *Ibid.* I. Abt. Originale, XXXVI. 465.

Gamaleïa (1892), *Les poisons bactériens.* Paris.

Ganghofner (1889), Article: "Diphtherie" in *Penzoldt-Stinzing.* First Supplement vol., Jena.——**(1897)**, *Serumbehandlung bei der Diphtherie.* Sammelforschung. Jena.——**(1902)**, Zur bacteriologischen Diagnose der Diphtherie. *Prag. med. Woch.*, p. 172.

Garratt, G. C. (XII. 1904), On acute diphtheritic whitlow in persons apparently healthy. *St Bartholomew's Hosp. Journ.*, XII. 35.——**(1905)**, The early sequelae of severe faucial diphtheria and their treatment. *St Bart.'s Hosp. Reports*, XL. 41.

Garratt, G. C. and Washbourn, J. W. (15. IV. 1899), A systematic bacteriological examination of the fauces in Scarlet Fever as a means of preventing post-scarlatinal diphtheria. *Brit. Med. Journ.*, I. 893.

Gaston, P. (1893), *Du Foie infectieux.* Asselina and Honzeau, 276. Paris.

Gauss, C. J. (24. I. 1902), Babes Ernst'sche Körperchen und Virulenz bei Bakterien. *Centralbl. f. Bakt.*, XXXI. 92.

Gayton, W. (26. V. 1894), A case of paralysis following diphtheria of the genitals only. *Lancet*, I. 1301.

Gehrke, W. (14. II. 1899), Ueber das Verhalten des Diphtheriebacillus in Wässern und auf Nährsubstraten unter dem Einfluss des direkten Sonnenlichtes. *Dissertation*, Greifswald, 1896. (Ref. *Centralbl. f. Bakt.*, XXV. 198.)

Geirsvold, M. (1903), Ueber das Vorkommen von Diphtheriebacillen bei gesunden Menschen. *Tidsskr. f. d. norske Loegeforening*, XXIV. 820. (Ref. *Baumgarten's Jahresber.*, XIX. 233.)

Genersisch, G. (1894), Bacteriologische Untersuchungen über die sogenannte septische Diphtherie. *Jahrb. f. Kinderheilk.*, XXXVIII. 233.

Gerber (31. VII. 1903), Die bakteriologische und die klinische Diagnose bei den fibrinösen Entzündungen der oberen Luftwege. *Berlin. klin. Wochenschr.*, XLII. 969.

Gerber and Podack (1895), Ueber die Beziehungen der sog. primären Rhinitis fibrinosa und des sog. Pseudodiphtheriebacillus zum Klebs-Loeffler'schen Diphtheriebacillus. *Deutsches Arch. f. klin. Med.*, p. 262. (Ref. *Centralbl. f. Bakt.*, XVII. 723.)

Gerhardt (1883), Ueber Diphtherie, ihre parasitäre Natur, Verhältniss des localen Processes zur allgemeinen Infection, Contagiosität, Therapie, und Prophylaxe. *Verhandl. d. Congr. f. innere Med.*, Wiesb., II. 125.

German (1905), Ueber "Cyllin." *Centralbl. f. Bakt.*, XXXVIII. 237.

Germano, E. (1897), Die Uebertragung von Infectionskrankheiten durch die Luft. *Zeitschr. f. Hyg. u. Infect.*, XXV. 439.

Ghirardini, P. (**1901**), Di una spinale forma di malattia contagiosa del piccione Considerata in rapporto alla difteria aviaria. *Il Nuovo Ercolani* (*Arch. quindic. di veterinaria e zootecnia*, vi). (Ref. *Baumgarten's Jahresber.*, xvii. 198.)

Ghisi, M. (**1749**), *Lettere mediche; la seconda contiene l'istoria delle angine epidemiche degli anni* 1747 *e* 1748. Cremona.

Gibier, P. (**1896**), Effets produits sur certains animaux par les toxines et les antitoxines de la diphtérie et du tétanos injectées dans le rectum. *Sem. Méd.*, xvi. 202.——(**1897**), Description d'un procédé permettant d'obtenir une toxine diphtérique extra-toxique. *Compt. rend. Soc. de Biol.*, iv. 392.

Gibson, R. B. (**1905–6**), The concentration of antitoxin for therapeutic use. *Journ. Biol. Chem.*, N. Y., i. 161.——(**1905**), The practical concentration of diphtheria antitoxin. *Am. Med.*, Philad., x. 915.

Gibson, R. B. and Collins, K. R. (**1906**), On the fractionation of agglutinins and antitoxin. *Proc. Soc. Exper. Biol. and Med.*, N. Y., iv. 15; also *Journ. of Biol. Chem.*, iii. 233.

Gilbrech, A. H. (**1906**), The contagiousness of diphtheria. *Journ. Amer. med. Assoc.* xlvii. 1940.

Gioelli, P. (**IX. 1903**), Sui bacilli pseudodifterici in rapporto all' eziologia e profilassi della difterite. *Policlinico.* (Ref. *Centralbl. f. Bakt.*, xxxv. 620.)

Gladin, G. (**1895**), Bolnitschnajagaseta Botkina, No. 20. (Ref. *Baumgarten's Jahresber.*, xi. 265.)

Glaessner, P. (**9. VI. 1900**), Ueber die Verwertbarkeit einiger neuer Eiweisspräparate zu Kulturzwecken. *Centralbl. f. Bakt.*, xxvii. 724.

Gläser (**1896**), Mitteilungen über 20. Jahre Diphtherie. *Zeitschr. f. klin. Med.*, xxx.

Gley, E. and Charrin, A. (**1893**), Influences héréditaires expérimentales. *Compt. rend. Soc. de Biol.*, p. 883.

Glücksman (**1895**), *Ueber die nach Anwendung des Diphtherieserums auftretende Exanthems.* (*Inaug. Dissert.* Leipzig.)

Glücksmann, S. J. (**1897**), Ueber die bakteriologische Diagnose der Diphtherie. *Zeitschr. f. Hyg. u. Infect.*, xxvi. 417.

Goadby, K. W. (**1900**), The utility of bacteriological examination in a diphtheria epidemic in a school, with special reference to the Poplar Union Schools at Forest Gate in 1898–1899. *Trans. Epidem. Soc.*, xix. 87.——(**24. I. 1903**), *Lancet*, i. 237. (Report of Meeting of Pathol. Soc. of London.)——(**1903**), *The Mycology of the Mouth.* Longmans, Green & Co.

Goldberg, S. (**1899**), Ueber die Ausscheidung des Tetanusgiftes durch Nierensecretion bei Experimentaltetanus. *Centralbl. f. Bact.*, xxvi. 547; also appeared in Russian in *Boln. Gaz. Botk.*, p. 903. (Ref. *Baumgarten's Jahresber.*, xv. 195.)

Goldscheider, A. (**1893**), Zur Lehre von den durch Streptokokken bedingten Erkrankungen. *Zeitschr. f. klin. Med.*, xiv. 681.

Goldscheider, E. (**1893**), Bakteriologische Untersuchungen bei Angina tonsillaris und Diphtherie. *Zeitschr. f. klin. Med.*, xxii. (Ref. *Centralbl. f. Bakt.*, xiv. 365.)

Golowkoff, A. J. (**1898**), Ueber Nährböden für die bakteriologische Diphtheriediagnose. [*Dissertation*, St Petersburg.] (Ref. *Centralbl. f. Bakt.*, xxv. 393.)

Golowkow, A. (**1895**), Zur Frage über die Lebensfähigkeit des Diphtheriebacillus Loeffler auf einigen Geweben. *Militärmed. Journ.*, ix. (Ref. *Centralbl. f. Bakt.*, xx. 319.)——(**1899**), *Wojenno-medic. shurn.*, No. 1, p. 187. (Ref. *Baumgarten's Jahresber.*, xv. 208.)

Gonin, J. (**1899**), De la nature microbienne des conjunctivitis. *Rev. méd. de la Suisse romande*, No. 2 and 3. (Ref. *Centralbl. f. Bakt.*, xxvi. 233.)

Goodall, E. W. (1896), On Post-Scarlatinal diphtheria in the Hospitals of the Metropolitan Asylums Board. *Trans. Epidemiol. Soc.*, xv. 68.——(1896), Diphtheria of the oesophagus. *Trans. Path. Soc.*, lxvii. 39.

Goodman, A. L. (1901), *New York Med. Record*, lix. 267.

Gordon, M. H. (1900–1), Further report on the bacteriology of scarlatina. *Thirtieth Ann. Rep. of the Local Government Board.* App. B, p. 353.——(1903), Report on Bacillus diphtheriae and microorganisms liable to be confounded therewith. *Ann. Rep. of Local Government Board*, 1901-2 (publ. 1903), pp. 418–439.

Gorham, F. P. (VII. 1901), Morphological varieties of Bacillus diphtheriae. *Journ. Med. Research*, vi. 201.

Gorini, C. (1895), Contributo alla diagnosi batteriologica della difterite. *Atti d. Assoc. med. lombarda.* (Ref. *Baumgarten's Jahresber.*, xi. 257.)

Goris (1893), Croup diphtérique d'emblée et abcès du Larynx. *Ann. d. Mal. de l'Oreille*, xix. (Quoted by Councilman, Mallory and Pearce, 4. xii. 1900.)

Gossage, A. M. (15. VIII. 1896), The influence of glycerine in culture media on the diphtheria bacillus. *Lancet*, ii. 458.

Gottstein, A. (1893), Die Contagiosität der Diphtherie. *Berlin. klin. Wochenschr.*, p. 594. (Ref. *Baumgarten's Jahresber.*, ix. 196.)——(1895), Ueber Todesfälle bei Anwendung des Diphtherieheilserums auftretende Exantheme. *Inaug. Dissert.*, Leipzig.——(1896), Die Bekämpfung der Diphtherie. *Berlin. klin. Wochenschr.*, p. 1125. (Ref. *Baumgarten's Jahresber.*, xii. 299.)——(1901), Diphtheriestatistik u.s.w. *Therap. Monatsh.*, xv.

Graham-Smith, G. S. (IV. 1902), The Measures taken to check the Diphtheria Outbreak of 1901 at Colchester. *Journ. of Hygiene*, ii. 170.——(IV. 1903), The Distribution of the Diphtheria Bacillus and the Bacillus of Hofmann in the throats of "Contacts" and Normal Persons. *Journ. of Hygiene*, iii. 216.——(IV. 1904), A Study of the Virulence of the Diphtheria Bacilli isolated from 113 Persons, and of 11 Species of Diphtheria-like Organisms, together with the Measures taken to check an Outbreak of Diphtheria at Cambridge, 1903. *Journ. of Hygiene*, iv. 258.——(1907), Some observations on "Swollen head" in turkeys. *Journ. of Agricult. Sci.*, ii. 227.

Grancher, J. (1897), Le diagnostic bactériologique et le diagnostic clinique de la diphtérie. *Bull. Méd. Par.*, xi. 239.

Gratia and Liénaux (1898), Bactériologie de la diphtérie aviaire. *La Semaine Méd.*, xviii. 206. (Report of Meeting of Acad. de Méd. de Belgique, 20. iv. 1898.)——(1898), Contribution à l'étude bactériologique de la diphtérie aviaire. *Bull. Acad. roy. de méd. de Belg.*, xii. 360.

Gray, H. (1896), Diphtheria of the Cat. *Journ. of Comp. Path. and Therap.*, ix. 46.

Green, J. O. (12. X. 1901), The bacteriology of Mastoiditis. *Journ. of Boston Soc. of Med. Sci.*, iii. 96. (Ref. *Centralbl. f. Bakt.*, xxx. 468.)——(1901), The primary infections in acute suppurations of the tympanum. *Journ. of Boston Soc. of Med. Sci.*, iii. 93. (Ref. *Centralbl. f. Bakt.*, xxx. 468.)

Grenet, A. and Lesné, E. (1898), Présence du bacille diphtérique dans les coryzas purulents non-pseudo-membraneux de l'enfant. *Arch. de Méd. d. enf.*, i. 449.

Gresswell (1890), *A contribution to the natural history of scarlatina.*

Griffith, A. S. (1901), The flora of the conjunctiva in health and disease. *Thompson Yates Labor. Rep.*, iv. pt. 1, p. 99.

Grigorieff, W. (1898), Examen bactériologique de la gorge et du nez chez des convalescents de diphtérie. *Arch. de Méd. d. enf.*, i. 467.

Grimes, L. A. (13. VIII. 1898), A case of membranous tracheitis and laryngitis without the presence of diphtheritic bacilli. *Lancet*, ii. 409. (Letter.)

Grixomi, G. (1896), Sulla presenza di bacilli simildifterici nelle otiti purulente; cura siero-terapica. *Riforma Med.*, iii. No. 151. (Ref. *Baumgarten's Jahresber.*, xii. 268.)

Gromakowsky, D. (**9. VIII. 1900**), Die differentielle Diagnose verschiedener Arten der Pseudodiphtheriebacillen und ihr Verhältniss zur Doppelfärbung nach M. Neisser. *Centralbl. f. Bakt.*, xxviii. 136.

Gross, H. W. (**1897**), The Klebs-Loeffler bacillus in apparently normal throats and noses. *University Med. Mag.*, ix. 45. (Ref. *Baumgarten's Jahresber.*, xiii. 309.)

Grosz (**1902**), Die antidiphtherische Serumtherapie. *Ungar med. Presse*, vii.

Guder (**1895**), Beiträge zur Conjunctivitis diphtheritica und deren Bedeutung in sanitätspolizeilicher Hinsicht. *Zeitschr. f. Medizinalb.*, No. 1. (Ref. *Centralbl. f. Bakt.*, xvii. 482.)

Guelpa (**1892**), Contribution à l'étude bactériologique de la diphtérie. *Bull. et mém. Soc. de Thérap.*, p. 207.

Guérin, C. (**15. I. 1903**), Sur la non-identité de la diphtérie humaine et de la diphtérie aviaire. *Rec. de Méd. Vétér.*, x. 20.——(**XII. 1902**), La Diphtérie aviaire. *Ann. de l'Inst. Pasteur*, xv. 941.——(**30. XI. 1903**), Sur la vaccination contre la diphtérie aviaire. *Rec. de Méd. Vétér.*, x. 511.

Guinochet, E. (**1892**), Contribution à l'étude de la toxine du bacille de la diphtérie. *Arch. de méd. expér. et d'anat. path.*, iv. 487.

Guthmann, H. (**1896**), Ueber die bakteriologische Diagnose der Diphtherie. [*Inaugural Dissertation.* Strassburg, i. E.] (Ref. *Centralbl. f. Bakt.*, xxi. 549.)

Haas (**1894**), *Contribution à l'étude de l'étiologie générale de la diphtérie.* Paris. (Ref. *Hyg. Rundsch.*, iv. 1070.)

Hadley, P. B. (**V. 1907**), The growth and toxin production of bacillus diphtheriae upon proteid-free media. *Journ. of Infect. Dis.*, Supplement 3, 95.

Hála, A. (**1900**), Ein Fall von Eiterung mit Diphtheriebacillenbefund. *Wiener klin. Rundsch.*, p. 673. (Ref. *Baumgarten's Jahresber.*, xvi. 194.)

Hallé (**1899**), Recherches bactériologiques sur le canal génital de la femme. *Ann. de Gynécol. et d'Obstétr.*, li. (Ref. *Centralbl. f. Bakt.*, xxvi. 645.)

Hallier, E. (**1868**), Die pflanzlichen Parasiten des menschlichen Körpers. Leipzig.

Hallwachs (**1897**), *Ueber die Myocarditis bei Diphtherie* [*Dissert.* Leipzig].

Hamilton, A. (**5. XI. 1904**), The question of virulence among the so-called pseudo-diphtheria bacilli. *Journ. of Infect. Dis.*, i. 690.——(**15. VI. 1907**), The opsonic index and vaccine therapy of pseudo-diptheritic otitis. *Journ. of Infect. Dis.*, iv. 313.——(**15. VI. 1907**), Pseudo-diphtheria bacilli as the cause of suppurative otitis, especially the post-scarlatinal. *Journ. of Infect. Dis.*, iv. 326.

Hamilton, A. and Horton, J. M. (**2. III. 1906**), Further studies on virulent pseudo-diphtheria bacilli. *Journ. of Infect. Dis.*, iii. 128.

Hammerschmidt (**1906**), Diphtheriebazillen im Eiter. *Ztschr. f. Hyg.*, liii. 504.

Hansemann (**1895**), Ueber die Beziehungen des Loeffler'schen Bacillus zur Diphtherie. *Arch. f. path. Anat.*, cxxxix. (Ref. *Centralbl. f. Bakt.*, xvii. 564.)

Hardman, W. (**16. II. 1895**), Another ready means of transmitting diphtherial and other discharges. *Brit. Med. Journ.*, i. 387.

Harmer, L. (**1900**), Untersuchungen über den Tonsillotomiebelag und seine etwaigen Beziehungen zum Diphtheriebacillus. *Wien. klin. Wochenschr.*, xiii. 846.

Harrington, C. (**27. VI. 1907**), An outbreak of diphtheria due to infected milk. *Boston Med. and Surg. Journ.*, clvi. 848.

Harrison, F. C. (**1903**), The supposed identity of human and avian diphtheria. *Public Health Rep. a. Papers of the Amer. Publ. Health Assoc.*, xxviii. 543.

Harrison, W. S. (**23. III. 1907**), [Diphtheria-like bacillus from the ear]. *Lancet*, i. 806.

Hart (**1859**), *Diphtheria, its history, progress, symptoms and treatment.* London.

Hartmann, F. (1871), Ueber Croup und Diphtheritis der Rachenhöhle, Exudat und Eiterbildung. *Virch. Arch.*, lii. 240.

Hartung (1896), Die Serum Exantheme bei Diphtherie. *Jahrb. f. Kinderheilk.*, xlii.

Hasslauer, W. (22. XII. 1902), Die Bakterienflora der gesunden und kranken Nasenschleimhaut. *Centralbl. f. Bakt.*, xxxiii. 47.——(1905), Die Mikroorganismen der gesunden und kranken Nasenhöhle und Nasennebenhöhlen. *Centralbl. f. Bakt.* (Abt. 1, Referate), xxxvii. pp. 1, 97.

Hassenstein, W. (1899), Ungewöhnliche Formen diphtherischer Erkrankungen, übertragen durch eine Hebamme. *Deutsche med. Wochenschr.*, p. 406.

Hau (1900), *Lyon Méd.*, No. 4. (Ref. *Amer. Journ. Med. Sci.*, cxx. 351.)

Haultain, F. W. (26. VI. 1897), Puerperal diphtheria. *Lancet*, i. 1745 (Report of Meeting of Edinboro' Obstet. Soc., 9, vi.)

Hayem, G. (1870), Études sur les Myosites symptomatiques. *Arch. de Physiol.*, iii. 81, 101, 269, 291, 422, 435, 475, 489, 569, 585.

Hayward, J. A. (1894), The diagnosis of diphtheria. *St Barth's. Hosp. Rep.*, xxx. 139.——(1895), The cultivation of the diphtheria bacillus on hydrocele fluid with remarks on an examination of thirty cases. *Trans. Path. Soc. of London*, xlvi. 291.

Head, G. D. and Wilson, L. B. (1899), A case of suspected rabies with isolation of Bacillus diphtheriae from the central nervous system. (Clinical Report.) *Journ. of Exper. Med.*, iv. 451.

Heaven, C. (4. I. 1902), Carrier cases of nose. *Lancet*, i. 55 (Letter).

Hector, C. M. (VI. 1905), Neisser's stain in the diagnosis of diphtheria. *New Zealand Med. Journ.*, iv. 175.

Heelis, R. and Jacob, F. H. (10. III. 1906), A series of four cases of cutaneous diphtheria. *Brit. Med. Journ.*, i. 556.

Heinersdorff, H. (1898), Ueber das Vorkommen den Diphtheriebacillen ähnlichen Mikroorganismen (Xerosebacillen, Septirterbacillen, Bacilles en massue, etc.) im menschlichen Conjunctivalsack, speciell auf der normalen Conjunctiva nebst einem Beitrage zur Frühdiagnosis der Diphtherie. *Arch. f. Ophthal.*, xlvi., 1.——(1898), Zur Schnelldiagnose der Diphtherie, speciell der Diphtherie der Conjunctiva. *Centralbl. f. Bakt.*, xxiii. 397.

Heinz, R. (1904), *Handbuch der experimentellen Pathologie und Pharmakologie.* G. Fischer; Jena, i. 898.

Hellström, F. E. (14. II. 1899), Zur Kenntniss der Einwirkung kleiner Glukosemengen auf die Vitalität der Bakterien. *Centralbl. f. Bakt.*, xxv. pp. 170, 217. ——(20. XII. 1899), Erwiderung auf einige Bemerkungen von Dr. Th. Madsen gegen die von mir vertretenen Ansichten betreffs der Wachstumserscheinungen. *Centralbl. f. Bakt.*, xxvi. 694.

Hellström, G. (1894), Fatorer i difteriens etiologie, särskilt stafylokockenas betydelse. *Hygiea*, Hft. 7, p. 46. (Ref. *Baumgarten's Jahresber.*, x. p. 227, 1895, and *Centralbl. f. Bakt.*, xvii. 37.)

Hellström, T. (1894), Jakttagelser angoaende difteribacillens fortvaro hos difteripatienter. Stockholm: F. W. Warfvinge, etc. [*Festd. Med. Doct.*], p. 37.

Henke, F. (1898), Die experimentelle Erzeugung von Diphtherie bei Tieren durch die Loeffler'schen Diphtheriebacillen. *Arb. a. d. pathol.-anat. Instit. Tübingen*, ii. (3) p. 321. (Ref. *Centralbl. f. Bakt.* (1898), xxiv. pp. 452–454 (Morgenroth).)

Hennig, A. (1896), Ueber den Werth des Diphtheriebacillus und über die Resultate meiner Diphtheriebehandlungsmethode. *Therap. Wochenschr.*, iii. 389.——(1896), Welchen Werth hat der Diphtheriebacillus in der Praxis? *Samml. klin. Vortr.*, n. F., No. 157.——(1897), Ueber chronische Diphtherie. *Wien. med. Wochenschr.*, p. 1605.

Herbold, O. (7. V. 1891), Beobachtungen bei einer kleinen Hausepidemie von Diphtheritis faucium. *Deutsche med. Wochenschr.*, xvii. 647.

Héricourt, J. and Richet, C. (1888), Sur un microbe pyogene et septique (Staphylococcus pyosepticus) et sur la vaccination contre ses effets. *Compt. rend. Acad. d. Sci. Paris*, cvii. 690.——(1889), Influence de la transfusion péritoneale du sang de chien sur l'évolution de la tuberculose chez le lapin. *Compt. rend. Soc. de Biol.*, 157.

Herpin (*see* Jaffé).

Herrera, P. de (1615), De essentia, causis, nofis praesagiis, curatione et praeventione faucium et gutturis anginosorum ulcerum morbi suffocantis, Garrotillo, etc. Matriti.

Hesse, B. (1893), Beiträge zur path. Anatomie des Diphtherieherzens. *Jahrb. f. Kinderheilk.*, xxxvi. 19–49.

Hesse, W. (1894), Zur Diagnose der Diphtherie. *Zeitschr. f. Hyg.*, xviii. 500.——(25. VI. 1896), Die bakteriologische und klinische Diagnose "Diphtherie." *Deutsche med. Wochenschr.*, xxvi. 419.

Heubner, O. (1883), *Die experimentelle Diphtherie.* Gekrönte Preisschrift. Leipzig.——(1889), Ueber die diphtherische Membran. *Verhandl. d. Congr. f. inn. Med.* Wiesbaden, 468–476.——(1890), Bemerkungen zur Frage der Scharlachdiphtheritis und deren Behandlung. *Jahrb. f. Kinderheilk.*, xxi.——(1892), Ueber Diphtherie. *Schmidt's Jahrbüch. der ges. Medizin*, ccxxxvi. (Ref. *Centralbl. f. Bakt.*, xiii. 729.)——(1895), *Klinische Studien über die Behandlung der Diphtherie.* 124 pp., 8^0, Leipzig.

Hewitt, C. N. (1898), Diphtheria: suggestions for its prevention and limitation. *Proc. Amer. Pub. Health Assoc.*, xxiv. 88.

Hewlett, R. T. (4. II. 1896), Diphtheria bacilli in a cultivation from the throat 22 weeks after the primary attack; bacilli markedly virulent. *Trans. Path. Soc.*, xlvii. 360.——(1899), On Neisser's diagnostic stain for the diphtheria bacillus. *Trans. Jenner Inst. Prev. Med.*, 2nd Ser., p. 201.——(1900), A preliminary note on the indol-like reaction given by cultures of the diphtheria and pseudo-diphtheria bacilli. *Trans. Path. Soc.*, li. 137.——(5. III. 1901), The Indol-like reaction given by cultures of the diphtheria and pseudo-diphtheria bacillus. *Trans. Path. Soc. of United Kingdom*, lii. 113.——(1902), The non-absorption of Antitoxin from the stomach and rectum. *Trans. Path. Soc. of London*, liii. 220.——(9. VII. 1904), The pseudo-diphtheria bacillus. (Report on progress of researches. Scientific Grants Committee of Brit. Med. Assoc.) *Brit. Med. Journ.*, Supplement, p. 16.

Hewlett, R. T. and Barton, G. S. (1907), The results of a chemical, microscopical and bacteriological examination of samples of London milk. *Journ. of Hygiene*, vii. 22.

Hewlett, R. T. and Knight, E. (1897), The "so-called" Pseudo-diphtheria bacillus and its relation to the Klebs-Loeffler bacillus. *Trans. Brit. Inst. Prev. Med.*, 1st Series, p. 7.

Hewlett, R. T. and Murray, H. M. (15. VI. 1901), A common source of diphtherial infection and a means of dealing with it. *Brit. Med. Journ.*, i. 1474.

Hewlett, R. T. and Nolan, H. (1. II. 1896), The results of Bacteriological examination of 1000 cases of suspected diphtheria. *Brit. Med. Journ.*, i. 266.

Heymanns, J. F. (1898), Sur la disparition du sang des poisons injectés. *Bull. de l'Acad. Roy. de Belg.*, xii. 751.

Hibbard, C. M. (1898), Heart complications in diphtheria. *Med. and Surg. Rep. Boston City Hosp.*, 9th Series, pp. 33–51.

Hibler, E. v. (23. I. 1896), Ueber das konstante Vorkommen von Spaltpilzeinschlüssen in den Zellen bei Eiterungsprozessen des Menschen nebst experimentellen Beiträgen zur Kenntniss und diagnostischen Bedeutung solcher Befunde. *Centralbl. f. Bakt.*, xix. 33, 113.

Higley, H. A. (1. IV. 1905), The rapid bacteriological diagnosis of diphtheria. *Med. Rec.*, lxvii. 492.

Hilbert (1899), Ueber die Steigerung der Giftproduktion der Diphtheriebacillen bei Symbiose mit Streptokokken. *Zeitschr. f. Hyg.*, xxix. 157.

Hildebrandt, G. (1888), Experimentelle Untersuchungen über das Eindringen pathogener Mikroorganismen von den Luftwegen und der Lunge aus. *Beiträge z. pathol. Anat. u. Physiol.* herausg. v. Ziegler u. Nauwerck, ii. 143. (Ref. *Baumgarten's Jahresber.*, iv. 378.)

Hill, H. W. (X. 1898), On the regulation requiring two consecutive negative cultures for the release of diphtheria cases. *Journ. Mass. Assoc. of Boards of Health*, viii. 7 pp. repr.——**(I. 1899)**, Branching forms of Bacillus diphtheriae. *Journ. of Boston Soc. of Med. Sci.*——**(10 I. 1900)**, Branching diphtheria bacilli. *Journ. of Boston Soc. of Med. Sci.*, iv. 78.——**(1901)**, Notes on the morphology of B. diphtheriae. *Ann. Rep. Boston Board of Health.*——**(7. III. 1901)**, The interpretation of bacteriological findings in diphtheria diagnosis. *Boston Med. and Surg. Journ.*, cxliv. 226.——**(I. 1902)**, Branching forms in bacteria with special reference to B. diphtheriae. *Journ. Med. Research*, vii. 115.——**(XII. 1902)**, The distribution of B. diphtheriae and B. tuberculosis in rooms occupied by patients suffering from these diseases. *Proc. Amer. Pub. Health Assoc.*, xxviii. 209.——**(16. V. 1903)**, Vorläufige Mitteilung über chromogene Kulturen von B. diphtheriae. Orig. Ref. in *Centralbl. f. Bakt.*, xxxiii. 276. ——**(15. XII. 1904)**, Innocent cases reported as diphtheria. *Boston Med. and Surg. Journ.*, cli. 654.

Hill, H. W. and Rickards, B. R. (XII. 1902), Notes on Morphology. *Proc. Amer. Pub. Health Assoc.*, xxviii. 479.

Hillary, W. (1766), Observations on the changes of the air and the concomitant epidemical diseases in the Island of Barbadoes. London.

Hillier, T. (I. 1859), On diphtherite. *Med. Times and Gaz.*, xviii. 107.

Hirsch, A. (1886), Die Organkrankheiten vom historisch-geographischen Standpunkte und mit besonderer Berücksichtigung der Aetiologie bearbeitet. In his *Handbuch der historisch-geographischen Pathologie*, Pt. iii., 48. Stuttgart. ——**(1886)**, Handbook of Geographical and Historical Pathology. (New Sydenham Society), London (translation of above).

Hiss, P. H. and Atkinson, J. P. (1900), Serum globulin and diphtheric antitoxin—a comparative study of the amount of globulin in normal and antitoxic sera, and the relation of the globulins to the antitoxic bodies. *Journ. of Exper. Med.*, v. 47.

Hodgetts, C. A. (1895), Management of diphtheria epidemics in rural districts. *Proc. Amer. Pub. Health Assoc.*, xx. 89.

Hofmann-Wellenhof, G. v. (1888), Untersuchungen über den Klebs-Loeffler'schen Bacillus der Diphtherie und seine pathogene Bedeutung. *Wien. med. Wochenschr.*, xxxviii. 65, 108. (Also ref. *Baumgarten's Jahresber.*, iii. 245.)

Holmes, J. D. E. (31. III. 1904), An outbreak of diphtheria associated with a similar disease among fowls and a vesicular eruption on the udders of cows. *Journ. of Comp. Path. and Therap.*, xvii. i.

Holst, P. F. (1893), *Norsk Magaz. Laegeridenskaben*, p. 325. (Ref. *Baumgarten's Jahresber.*, ix. 187.)

Home, F. (1809), *An inquiry into the nature, cause, and cure of the croup*, Edinburgh, 1765. Aus dem Englischen übersetzt von Mohr, mit Vorrede und Anmerkungen von J. A. Albers. Bremen, 1809.

Homolle (1875), *Contributions à l'étude de la diphtérie.* Lille.

Horne, J. W. (16. IX. 1905), *Brit. Med. Journ.*, ii. p. 651.

Horton-Smith (1897), On the bacteriology of acute broncho-pneumonia. *St Barthol. Hosp. Rep.*, xxxiii. 25.

Houston, A. C. (1898), Procedures preliminary to inoculations of soils with the cholera microbe and the bacillus of diphtheria with a view to ascertaining the ultimate fate of these organisms. *Local Government Board Rep.*, 1897-8, App. B, No. 2, xxvii. p. 296.

Howard, W. T. (**IV. 1893**), A case of acute *ulcerative endocarditis* due to the Bacillus diphtheriae. *Johns Hopkins Hosp. Bull.*, iv. 32–33.——(**XII. 1897**), The influence of cow's milk in the spread of diphtheria. *Amer. Journ. of Med. Sci.*, cxii. 629.

Howard, W. T. and Ingersoll, J. M. (**V. 1898**), A contribution to our knowledge of the etiology of inflammations of the accessory sinuses of the nose. *Amer. Journ. of Med. Sci.*, cxv. 520.

Hubbard, T. (**14. X. 1899**), Report of cases of peritonsillar abscess associated with diphtheria. *New York Med. Journ.*, lxx. 549.

Hueter, C. (1873), *Die allgemeine Chirurgie*, p. 205. [Leipzig.]

Hueter, C. and Crudeli, T. (1868), Pilzsporen in den Geweben und im Blut bei Gangraena diphtheritica. *Centralbl. f. d. med. Wissensch.*, vi. 252.

Hugenschmidt (1896), Étude expérimentale des divers procédés de défense de la cavité buccale contre l'invasion des bactéries pathogènes. *Ann. Inst. Pasteur*, x. 545.

Hugounenq and Doyon (1896), *La Province Méd.* (Quoted by Kossel.)——(**18. IV. 1896**), A propos de la culture du bacille de Loeffler en milieu chimique définé. *Compt. rend. Soc. Biol.*, Ser. X., iii. 401.

Huguenin, P. C. (1888), La myocardite infectieuse diphtérique. *Rev. de Méd.*, viii. ——(**1890**), *Étude anatomo-pathologique et clinique de la myocardite infectieuse diphtérique.* Paris, 104 pp.

Hunsberger, J. N. and Bergey, D. H. (**24. V. 1902**), An unusual case of diphtheria. *Philad. Med. Journ.*, ix. 943.

Hunt, B. (1895), The pathology of diphtheria. *Brit. Med. Journ.*, i. 532. (Meeting of Path. Soc. of London.)

Hunt, J. M. (**22. X. 1898**), On the relation of fibrinous rhinitis to diphtheria. *Brit. Med. Journ.*, ii. 1249.

Hutchens, H. J. (**III. 1906**), Diphtheria: its prevention and treatment. *Public Health.*

Huxham, J. (1784), *Opera physico-medica.* Editio nova. Lipsini. Tomus tertius, pp. 93, 103 (1751 et 1753).

Ibrahim, J. (1905), Ueber Schutzimpfungen mit Diphtherieheilserum. *Deutsche med. Wochenschr.*, xxxi. 412.

Ide, U. and Lemaire, A. (1899), Étude sur la réparation de l'antitoxine diphtérique dans les groupements albumineux de sérum. *Arch. internat. de Pharmacodyn. et de Thér.*, vi. 287.

Igl (1896), Oesterr. Sanitätswesen, No. 16. (Ref. *Hyg. Rundsch.*, vi. 563.)

Jaccoud (1893), Les angines pseudo-membraneuses à pneumocoques. *La Semaine Méd.*, No. 44.——(**VII. 1893**), *La Semaine Méd.*, p. 346.

Jacob, F. H. (**16. IX. 1905**), *Brit. Med. Journ.*, ii. 651.

Jacobi, A. (1884), Diphtheria spread by adults. *New York Med. Journ.*, xl. 344.

Jacques (**III. 1892**), De la diphtérie et de sa nature bacillaire au point de vue du traitement. *Rev. mens. d. malad. de l'enfance.* (Ref. *Centralbl. f. Bakt.*, xiii. 735.)

Jaffé (1843), Die Diphtherie in epidemiologischer und nosologischer Beziehung. *Schmidt's Jahrb. der ges. Med.*, cxiii. pp. 101, 103 [quotes Herpin].

Jakowski (**1894**), Kilka uwag w kwestyi badania bton dyftery-tycznych. *Gazeta Lekarska*, p. 1178. (Ref. *Baumgarten's Jahresber.*, x. 185.)——(**1895**), Einige Bemerkungen zur bakteriologischen Untersuchung der Diphtheriemembranen. *Gazeta Lekarska*, p. 1878. (Ref. *Centralbl. f. Bakt.*, xvii. 897.)

Janson, C. (**IV. 1893**), 100 Fälle von pseudomembranöeen Angina, besonders auf Diphtheriebacillen untersucht. *Hygiea.* (Ref. *Centralbl. f. Bakt.*, xiv. 143.)

Jensen, F. (**1897**), *Centralbl. f. inn. Med.*, No. 19. (Ref. *Amer. Journ. Med. Sci.*, cxv. 467.)

Jervey, J. W. (**2. VI. 1902**), Some desultory remarks concerning diphtheria. *New York Med. Journ.*, lxxi. 863.

Jessen, F. (**1897**), Ueber prolongirte Diphtherie. *Centralbl. f. innere Med.*, xviii. 449; also *Münch. med. Wochenschr.*, 1897.

Jessop, W. H. H. (**1895**), Two cases of membranous conjunctivitis in which Loeffler's bacillus was found. *Trans. Ophthal. Soc. of United Kingdom*, xv. 55. ——(**22. III. 1902**), *Brit. Med. Journ.*, i. 720 (report of meeting of Ophthal. Soc. of United Kingdom).

Jéz, L. (**1895**), Ueber das Vorkommen von Diphtheriebacillen im Herpes labialis in Verlaufe von Rachendiphtherie. *Przgelad Lekarski*, No. 35. *Centralbl. f. inn. Med.*, xvii. 1896, p. 163.

Jodin, N. (**1859**), *De la nature et du traitement du croup et des angines couenneuses.* Paris.

Johannessen (**1895**), Ueber Injectionen mit antidiphtheritischen Serum und mit reinem Pferdeserum. *Deutsche med. Wochenschr.*, xxi.

Johns, J. R. (**1899**), Clinical diphtheria. A summary of investigations concerning the diphtheria bacillus, the toxin and antitoxin, including diagnosis, prognosis and treatment of the disease. *Philad. Monthly Med. Journ.*, i. 179.

Johnson, A. N. V. and Goodall, E. (**16. VIII. 1902**), A case of acute mental disorder, probably toxic in origin, secondary to influenza. *Lancet*, ii. 439.

Johnston, W. (**IX. 1891**), Forms of true diphtheria which simulate simple catarrhal angina. The so-called diphtheritic angina sine membrana. *Montreal Med. Journ.* (Ref. *Centralbl. f. Bakt.*, xii. 392.)——(**1891**), Notes on the bacteriological study of diphtheria. *Bacteriol. World*, Columbia, Mo., i. 691–706.——(**IX. 1891**), Notes on the bacteriological studies of diphtheria. *Montreal Med. Journ.*——(**10. XII. 1892**), A new method for the culture of diphtheria bacilli in hard-boiled eggs. *Philad. Med. News*, lxi. 659. (Ref. *Baumgarten's Jahresber.*, viii. 186.)

Johnstone, J. (**1779**), *A treatise on the malignant angina; or putrid and ulcerous sore throat, to which are added some remarks on the angina trachealis.* Worcester.

Joos, A. (**1896**), Une nouvelle méthode pour la diagnostic bactériologique de la diphtérie. *Journ. méd. de Bruxelles*, No. 19. (Ref. *Centralbl. f. Bakt.*, xxii. 194.) ——(**31. III. 1899**), Untersuchungen über Diphtheriediagnose. *Centralbl. f. Bakt.*, xxv. pp. 296, 351.

Jordan, E. O. (**18. II. 1899**), The death-rate from diphtheria in the large cities of the United States. *Philad. Med. Journ.*

Jörgensen, A. and Madsen, Th. (**1902**), The fate of typhoid and cholera agglutinins during active and passive immunisation. *Contrib. from the Univ. Lab. for Med. Bacteriology to celebrate the inauguration of the State Serum Institute.* Copenhagen.

Josias, A. and Tollemer, J. (**8. X. 1902**), (Studies in Diphtheria). *La Méd. Moderne.* (Ref. *Med. News*, lxxxi. 938.)

Jump, H. D. (**11. I. 1902**), Duration of Immunity by Diphtheria Antitoxin. *Philad. Med. Journ.*, ix. 69.

Jurine, L. (**1816**), *Abhandlung über den Croup*, übersetzt von Heineken mit einer Vorrede von J. A. Albers. Leipzig.

Kahnert (1900), Ueber eine Gruppe klinisch und ätiologisch zusammengehöriger Fälle von chronischer Erkrankung der oberen Luftwege. *Deutsche med. Wochenschr.*, xxvi. 525.

Kanthack, A. A. (22. VIII. 1896), Metachromatism in diphtheria bacilli. *Lancet*, ii. 531.——(12. IX. 1896), Ueber verzweigte Diphtheriebacillen. *Centralbl. f. Bakt.*, xx. 296.

Kanthack, A. A. and Stephens, J. W. W. (28. III. 1896), A new and easy method of preparing Serum-Agar; an aid to the diagnosis of diphtheria. *Lancet*, i. 835.——(8. V. 1896), Ein neues und bequemes Verfahren zur Bereitung von Serum-Agar-Agar als Hilfmittel zur Erkennung der Diphtherie. *Centralbl. f. Bakt.*, xix. 609.——(1897), The escape of diphtheria bacilli into the blood and tissues. *Journ. Path. a. Bact.*, iv. 45.

Kanthack, A. A. and White, C. P. (1895), Bacteriological investigations on diphtheria. *St Barth. Hosp. Rep.*, xxxi. 87.

Kasansky, M. W. (10. II. 1899), Die Einwirkung der Winterkälte auf die Pest- und Diphtherie-bacillen. *Centralbl. f. Bakt.*, xxv. 122.

Kassel, K. (1898), Ein Fall von primärer isolierten Nasendiphtherie. *Therap. Monatsh.*, No. 10. (Ref. *Centralbl. f. Bakt.*, xxv. 151.)

Kassowitz (1902), Die Erfolge des Diphtherieheilserums. *Therap. Monatsh.*, xvi.

Kasztan, G. (1900), Beitrag zur Frage der Augendiphtherie. *Dissert.* Würzburg. (Ref. *Baumgarten's Jahresber.*, xvii. 193.)

Katzenstein, M. (1895), Ueber die sekundäre Veränderungen der Organe bei Rachendiphtherie. *Münch. med. Abhandlungen*, 47 pp.

Kauder, G. (1886), Zur Kenntnis der Eiweisskörper des Blutserums. *Arch. f. exper. Path. u. Pharmak.*, xx. 411.

Kayser, H. (1905), Diphtherie-antitoxinbestimmung bei Mütter und Neugeborenen. *Zeitschr. f. klin. Med.*, lxi. 17–20.

Kedrowski (1901), Ueber die Kultur der Lepraerreger. *Zeitschr. f. Hyg.*, xxxvii. 52.

Kellog, W. H. (III. 1901), Bacteriologic diagnosis of diphtheria. *Occidental Med. Times.* (Ref. *Baumgarten's Jahresber.*, xvii. 169.)

Kelsh (1903), La diphtérie humaine et la diphtérie aviaire. *Rev. d'hyg.*, xxv. 989, 1071.

Kempner, W. (1. V. 1896), Ein Beitrag zur bakteriologischen Diagnose der Diphtherie. *Hyg. Rundsch.*, vi. 409.

Kinyoun, J. J. (1894), Report on the treatment of diphtheria by antitoxic serum and notes on the prevention of diphtheria. The Abstract of *Sanit. Reports*, Washington, ix. No. 51.

Kitai, E. (1900), Zur Neisser'schen differentialdiagnostischen Färbung der Diphtheriebacillen (Russian). *Eshenedelnik*, No. 42. (Ref. *Baumgarten's Jahresber.*, xvii. 177.)

Kitasato, S. (1889), Die negative Indol Reaction der Typhusbacillen in Gegensatz zu anderen ähnlichen Bacillenarten. *Zeitschr. f. Hyg.*, vii. 513.

Klebs (1873), Beiträge zur Kenntniss der Mikrokokken. *Arch. f. exper. Pathol. u. Pharmakol.*, i. 31.——(1875), Beiträge zur Kenntniss der pathogenen Schistomykosen. *Ibid.* iv. 107, 207.——(1883), Ueber Diphtherie, ihre parasitäre Natur, Verhältniss des localen Prozesses zur allgemeinen Infection, Contagiosität, Therapie (Chirurgie), und Prophylaxie. *Verhandl. des Congr. f. inn. Med.*, Wiesbaden, ii. 125.——(1899), Diphtheria. *Journ. Amer. Med. Assoc.*, xxxiii. 1520.

Klein, E. (1888), On the etiology of diphtheria. *Local Govern't Board Report*, App. B, xviii. p. 425.——(1889), Further report on the etiology of diphtheria. *Local Govern't Board Report*, xix. 143.——(1890), Further report on the etiology of diphtheria. *Local Govern't Board Report*, 219.——(**14 & 29. IV. 1890**), Zur Aetiologie der Diphtherie. *Centralbl. f. Bakt.*, vii. 489, 521.——(**22. V. 1890**), A contribution to the etiology of diphtheria. *Proc. Roy. Soc.*, Lond., xlviii. 71.——(**13. VI. 1890**), Ein weiterer Beitrag zur Aetiologie der Diphtherie. *Centralbl. f. Bakt.*, vii. 785.——(**28. VI. 1890**), Nachtrag zum "Weiteren Beitrag zur Aetiologie der Diphtherie." *Centralbl. f. Bakt.*, viii. 7.——(**1893**), Report on the pathology and etiology of diphtheria. *Local Govern't Board Report* for 1891–92, App. B, p. 125.——(**1894**), The inoculation of milch cows with cultures of the Bacillus diphtheriae. *Journ. Pathol. a. Bact.*, ii. 428–446.——(**27. IV. 1894**), Ein weiterer Beitrag zur Kenntniss der intracellulären Bakteriengifte. *Centralbl. f. Bakt.*, xv. 598.——(**10. VIII. 1895**), The diagnostic value of the diphtheria bacillus. *Lancet*, ii. 363.——(**31. VIII. 1895**), A discussion on the doubtful cases of diphtheria. *Brit. Med. Journ.*, ii. 526.——(**1896-7**), Report on further observations of the microbes of Vaccinia and Variola. *Local Govern't Board Rep.*, App. B, No. 6, p. 267.——(**1898–9**), Report on the fate of pathogenic and other infective microbes in the dead animal body. *Local Govern't Board Report*, xxviii. 344.——(**31. V. 1899**), Zur Kenntniss des Schicksals pathogener Bakterien in der beerdigten Leiche. *Centralbl. f. Bakt.*, xxv. 737.——(**1899–00**), On the behaviour of certain pathogenic microbes in milk, cream and cheese. *Local Govern't Board Report*, App. B, No. 8, p. 577.——(**15. X. 1900**), Ueber zwei neue pyogene Mikrobien: Streptococcus radiatus und Bacterium diphtheroides. *Centralbl. f. Bakt.*, xxviii. 417.——(**I. 1901**), Pathogenic microbes in milk. *Journ. of Hygiene*, i. 85.——(**1903**), Ein neuer pathogener Mikrob, zur Gruppe der Diphtheriebacillen gehörig = Bakterium muris. *Centralbl. f. Bakt.*, xxxiii. 488.——(**24. I. 1903**), *Lancet*, i. 237. (Report meeting Pathol. Soc., London.)

Klemensiewicz, R. and Escherich, Th. (**II. 1893**), Ueber einen Schutzkörper im Blute der von Diphtherie geheilten Menschen. *Centralbl. f. Bakt.*, xiii. 153.

Klitine, J. (**1899**), De la leucocytose dans la diphtérie. *Arch. des sci. biol.*, vii. 366. (Ref. *Baumgarten's Jahresber.*, xv. 253.)

Knapp, A. (**XI. 1904**), The differentiation of Bacillus diphtheriae, Bacillus xerosis and Bacillus pseudo-diphtheriae by fermentation tests in the serum-water media of Hiss. *Journ. of Med. Research*, xii. 473.

Knorr, A. (**1898**), Das Tetanusgift und seine Beziehungen zum thierischen Organismus. *München. med. Wochenschr.*, xlv. 321.——(**1899**), In Behring's *Allg. Ther. d. Infektionskrankh.*, p. 989.

Kober, M. (**1899**), Die Verbreitung des Diphtheriebacillus auf der Mundschleimhaut gesunder Menschen. *Zeitschr. f. Hyg.*, xxxi. 433.

Kohn, B. (**10. I. 1903**), The causes of death in diphtheria. *American Med.*, v. 61.

Kohn, E. (**VIII. 1894**), Diphtheritis und Schulhygiene. *Wien. klin. Wochenschr.*, vii. 613, 632.——(**XI. 1902**), Ueber den antiseptischen Wert des Argentum colloidale Credé und seine Wirkung bei Infektion. *Centralbl. f. Bakt.*, xxxii. 732.

Kohos (**19. V. 1897**), Sur le développement tardiv du bacille diphtérique. *Compt. rend. Soc. Biol.*, Ser. X., iv. 522.

Kolisko, A. and Paltauf, R. (**1889**), Zum Wesen des Croups und der Diphtherie. *Wien. klin. Wochenschr.*, ii. No. 8, 147–149.

Komotzky (**II. 1902**), Recherches sur les lésions vasculaires provoquées par les toxines diphtériques. *Ann. l'Inst. Pasteur*, xvi. 156.

Koplik, H. (**III. 1894**), Acute lacunar diphtheria of the tonsils, with studies on the relation of the real to the Pseudo-bacillus diphtheriae. *New York Med. Journ.*——(**1896**), The rapid bacteriological diagnosis of diphtheria. *New York Med. Journ.*, lxiv. 147.——(**2. VIII. 1892**), Forms of true diphtheria which simulate simple catarrhal angina; the so-called diphtheritic angina sine membrana. *New York Med. Journ.*, lvi. 225.

Korchoune, S. (31 I. 1902), *Roussky Arch. Pathol., Klin. Med. i Bacteriol.* (Rev. *New York Med. Journ.*, lxxv. 654.)

Kossel, H. (1894), Ueber die Behandlung der Diphtherie des Menschen mit Diphtherieheilserum. *Zeitschr. f. Hyg.*, xvii. 489.——(1895), Untersuchungen über Diphtherie und Pseudodiphtherie. *Charité-Annalen*, xx. 576 (also ref. *Baumgarten's Jahresbericht*, xi. 262).——(**VI. 1896**), Zur Kenntniss des Diphtheriegiftes. *Centralbl. f. Bakt.*, xix. 977.

Kraus, R. (1899), Ueber den Einfluss erhöhter Körpertemperatur auf Intoxication und Immunisirung. *Arch. internat. de Pharmakodyn. et de thérap.*, vi. 345.——(1901), Ueber diagnostische Verwendbarkeit der specifischen Niederschläge. *Wien. klin. Wochenschr.*, xiv. 693.

Kraus, R. and Eisenberg, P. (1902), Ueber Immunisirung mit Immunsubstanzen. *Centralbl. f. Bakt.*, xxxi. 208.

Kraus, R. and Joachim, J. (1903), Zur Frage den passiven Immunisirung. *Wien. klin. Wochenschr.*, xvii.

Krause, F. (14. **VII.** 1904), Primäre Diphtherie eines Nierenbeckens durch Operation geheilt. *Deutsche med. Wochenschr.*, xxx. 1061.

Kresling, K. (1896), Die bakteriologische Untersuchung der diphtherieverdächtigen Halsbeläge. *Pharmaz. Zeitschr. f. Russland*, Petersburg. (Ref. *Centralbl. f. Bakt.*, xxiii. 557.)

Kretz, R. (1901), Ueber die Beziehungen zwischen Toxin und Antitoxin. *Zeitschr. f. Heilk.*, p. 137.

Krüger, S. (1895), Ueber die chemische Wirkung der Elektrolyse auf toxische und immunisirende Bacteriensubstanzen. *Deutsche med. Wochenschr.*, xxi. 331.

Kruse, W. and Pasquale, A. (1894), Untersuchungen über Dysenterie und Leberabscess. *Zeitschr. f. Hyg.*, xvi. 1.

Kuck, J. (1891), *Zur Kenntniss der diphtheritischen Albuminurie und Nephritis. Inaug. Diss.*, München.

Kühnau, W. (1897), Ueber Mischinfektionen mit Proteus bei Diphtherie der Halsorgane. *Zeitschr. f. klin. Med.*, xxxi. (Ref. *Centralbl. f. Bakt.*, xxi. 688.)

Kuprianow, J. (1894), Experimentelle Beiträge sur Frage der Immunität bei Diphtherie. *Centralbl. f. Bakt.*, xvi. 415.

Kurth, H. (1898), Ueber die Diagnose des Diphtheriebacillus unter Berücksichtigung abweichender Culturformen derselben. *Zeitschr. f. Hyg.*, xxviii. 409.

Kutschbert, and Neisser, E. (1883 and 1884), Zur Pathologie und Aetiologie der Xerosis epithelialis conjunctivae und der Hemeralopia idiopathica. *Breslauer ärztl. Zeitschr.*, v. 40 ; also *Deutsche med. Wochenschr.*, Nos. 21, 24.

Kutscher (1894), Der Nachweis der Diphtheriebacillen in den Lungen mehrerer an Diphtherie verstorbener Kinder durch gefärbte Schnittpräparate. *Zeitschr. f. Hyg.*, xviii. 167.——(1898), Ueber die Aetiologie einer im Verlauf von Rachendiphtherie entstandenen Otitis media. *Deutsche med. Wochenschr.*, xxi. 162.

Kyle, D. B. (1895), Fibrino-plastic exudates ; with reports of two cases of laminated fibrino-plastic rhinitis, including laboratory investigation. *New York Med. Journ.*, lxii. 5.

Labadie-Lagrave, F. (1873), *Des complications cardiaques du croup et de la diphtérie.* Paris.

Laboulbène, A. (1861), *Recherches cliniques et anatomiques sur les affections pseudo-membraneuses, productions plastiques, diphtériques, ulcéro-membraneuses, aphtheuses, croup, muguet, etc.* Paris.

Lack, H. L. (1899), Fibrinous or membranous rhinitis and its relation to diphtheria. *Med.-chirurg. Trans.*, lxxxii. 1.

Laird, A. T. (1903), Recent studies regarding the morphology of the diphtheria bacillus. *Albany Med. Ann.*, XXIV. 252.

Lambotte, U. (18. XII. 1901), Les sensibilisatrices des bacilles diphtériques et pseudo-diphtériques. *Centralbl. f. Bakt.*, XXX. 817.

Landman. (Quoted by Galli-Valerio, 1904.)

Langer (1902), Zur bakteriologischen Diagnose der Diphtherie. *Prag. med. Wochenschr.*, p. 173.

Langhans (1753), *Beschreibung verschiedener Merkwürdigkeiten des Siementales nebst genauerem Bericht über eine neue ansteckende Krankheit.* Zürich.

Laser, H. (1894), Ueber den Einfluss der Citronensäure auf den Diphtheriebacillus. *Hyg. Rundsch.*, IV. 102.

Laslett, E. E. (VI. 1902), The pathology of Post-diphtheritic paralysis. *Journ. Pathol. a. Bacteriol.*, VIII. 228.

Lawe, J. (III. 1859), Diphtheria (Letter to the Editor). *Lancet*, I. 250.

Lawson, A. (1899), The bacteriology of the normal conjunctival sac from a report of 200 cases, and its practical bearing on the utility of antiseptics in ophthalmic surgery. *Trans. Jenner Inst. Prev. Med.*, 2nd Ser., p. 56.

Laycock, T. (29. V. 1859), Clinical lecture on diphtheria as caused by Oidium albicans. *Med. Times a. Gaz.*, XVI. 547.

Leary, T. (1896), On an unusual pathogenic action of the diphtheria bacilli. *Med. a. Surg. Reports Boston City Hosp.*, p. 129.——(15. VI. 1897), Diphtheritic gastritis. *Journ. of Boston Soc. Med. Sci.*, I. 8.

Leber (1873), *Centralbl. f. d. med. Wiss.*, XI. No. 9.

Le Clerc (1898), De la diphtérie du scrotum; considérations générales sur la diphtérie de la peau. *Clinique*, XII. p. 65.

Ledingham, J. C. G. (1907), Notes on the leucocyte-reaction during the immunisation of the horse and goat with diphtheria toxin. *Journ. of Hygiene*, VII. 92.
——(1907), On the relation of the antitoxin to the globulin-content of the blood serum during diphtheria immunisation. *Ibid.* VII. 65.

Ledoux-Lebard (1893), Action de la lumière sur le bacille diphtérique. *Arch. de Méd. expér. et d'Anat. path.*, v. 779. (Ref. *Baumgarten's Jahresber.*, IX. 172.)

Lee, B. (1896), Infected Milk Supply. *Proc. Amer. Pub. Health Assoc.*, XXIV. 281.

Leegaard, F. (1903), Diphtheriebacillen bei gesunden Schulkindern. *Tidsskr. f. d. norske Laegeforening*, XXIV. 651. (Ref. *Baumgarten's Jahresber.* (1905), p. 233.)

Lefèvre, H. A. (1891), *Contribution à l'étude de la diphtérie.* Paris.

Le Gendre, P. and Pochon, G. (1895), Cas remarquable de persistance du bacille diphtérique dans le mucus avec variations de sa virulence. *Rev. d'Obstétr.*, VIII. 376. (Ref. *Baumgarten's Jahresber.*, XI. 193.)

Lehmann, K. B. and Neumann, R. (1896), *Atlas und Grundriss der Bakteriologie.* München.

Leick, B. (22. III. 1900), Primäre Diphtherie der Vulva. *Deutsche med. Wochenschr.*, XXVI. 196.

Leighton, M. O. (28. III. 1901), Infection by means of modelling clay. *Centralbl. f. Bakt.*, XXIX. 446.——(XI. 1901), *Pediatrics.* (Ref. *Amer. Med.*, III. 36.)

Leloir, H. (V.—VI., 1880), Contribution à l'étude de la structure et du développement des productions pseudo-membraneuses, sur les muqueuses et sur la peau. *Arch. de physiol. norm. et pathol.*, Paris, 2 Sér. XII. Année, p. 420.

Lemoine, G. H. (XII. 1895), Contribution à l'étude bactériologique des anginas non-diphtériques. *Ann. de l'Inst. Pasteur*, IX. 877.——(25. VI. 1897), Virulence du bacille de Loeffler dans ses rapports avec les formes cliniques de la diphtérie. *Soc. Méd. d. Hôpitaux.* (Ref. *La Sem. Méd.*, XVII. 249.)

Leonhardi (1893), Ueber Krupp, Diphtherie, und Scharlach. *Samml. klin. Vorträge von Volkman*, No. 55. (Ref. *Centralbl. f. Bakt.*, xiii. 730.)

Lépecq de la Clôture (1778), *Collection d'observations sur les maladies et constitutions épidémiques.* Rouen.

Le Play (1905), La Diphtérie à l'Hôpital des Enfants-Malades, Paris, xxii.

Lesieur, Ch. (**25. X.** and **3. XI. 1900**), Contribution à l'étude du diagnostic bactériologique de la diphtérie; recherches sur la fréquence et les caractères des bacilles pseudo-diphtériques. *Province Méd.*——(**1901**), Les bacilles dits pseudo-diphtériques—I. mémoire: Leur rôle en pathologie humaine. *Journ. de Phys. et de Pathol. génér.*, iii. (Ref. *Baumgarten's Jahresber.*, xviii. 218.)——(**1901**), De l'agglutination des bacilles dits pseudo-diphtériques par le sérum antidiphtérique. *Compt. rend. Soc. Biol.*, lii. 819.——(**27. VII. 1901**), Production de paralysies chez la cobaye par les bacilles dits "pseudo-diphtériques." *Ibid.* liii. 817.

Letzerich, L. (**20. I. 1869**), Beiträge zur Kenntnis der Diphtheritis. *Virchow's Arch.*, xlv. 327.——(**19. III. 1869**), Zur Kenntnis der Diphtheritis. *Virchow's Arch.*, xlvi. 229.——(**8. II. 1871**), Diphtheritis und Diphtherie. *Virchow's Arch.*, lii. 231.——(**10. IX. 1873**), Die Entwickelung des Diphtheriepilzes. *Virchow's Arch.*, lviii. 303.——(**14. X. 1874**), Die locale und allgemeine Diphtherie. *Virchow's Arch.*, lxi. 457.——(**6. XII. 1876**), Experimentelle Untersuchungen. *Virchow's Arch.*, lxviii. 519.——(**1880**), *Arch. f. exper. Pathol. u. Pharmakol.*, xii. 354.

Leverett, J. (**15. IX. 1900**), A few cases of diphtheria and what they have taught me. *Philad. Med. Journ.*, vi. 510.

Levin, E. (1901), Ueber Diphtherie- u. Pseudodiphtheriebacillen. *Hygiea*, N.F. 1, p. 260. (Ref. *Vierteljahrschr. f. öffentl. Gesundheitspfl.*, xxxiv., suppl. p. 157.)

Levy (1897), Ein neues aus einem Falle von Lepra gezüchteten Bakterium aus der Klasse der Tuberkelbacillen. *Arch. f. Hyg.*, xxx. 168.

Levy, E. and Fickler, H. (**28. VI. 1900**), Ueber ein neues pathogenes keulenförmiges Bakterium der Lymphe (Corynebacterium lymphe vaccinalis). *Deutsche med. Wochenschr.*, xxvi. 418.

Lewandowski (1890), *Deutsche med. Wochenschr.*, p. 1186. (Quoted by Hewlett, 1900.)

Lewandowsky, F. (1904), Die Pseudodiphtheriebacillen und ihre Beziehungen zu den Diphtheriebacillen. *Centralbl. f. Bakt.*, xxxvi. 336, 472.

Lewis, P. A. (**19. XII. 1906**), Diphtheria-toxon paralysis in the guinea-pig. *Journ. med. Research*, xv. 469.

Leyden, E. (1882), Ueber die Herzaffectionen bei der Diphtherie. *Zeitschr. f. klin. Med.*, iv. 334–352.

Lichtheim (1883), *Verhandl. des II. Congr. f. inn. Med.* Wiesbaden.

Lichtwitz (**14. III. 1896**), Présence fréquente du bacille de Loeffler sur la plaie opératoire après l'ablation de l'amydale avec l'anse électrothérmique. Innocuité du bacille dans ces cas. *Compt. rend. Soc. de Biol.*, iii. 307.

Lie, H. (1900), Om den bakteriologiske difteridiagnose. *Med. Revue*, xvii. 129. (Ref. *Baumgarten's Jahresber.*, xvi. 198.)

Linossier and Lemoine (1903), Action néphrotoxique des Injections des sérums normaux. *Compt. rend. Soc. de Biol.*, lv. 515.

Lipstein, A. (1903), Ueber Immunisirung mit Diphtheriebacillen. *Centralbl. f. Bakt.*, xxxiv. 421.

Lissner, M. (1899), Ein Fall diphtherischer Infection eines Neugeborenen. *Arch. f. Kinderheilk.*, xxvi. 371. (Ref. *Baumgarten's Jahresber.*, xv. 257.)

Lister, T. D. (**29. X. 1898**), *Brit. Med. Journ.*, ii. 1338. Also letter, *ibid.* 5. xi. 1898, p. 1464.

Livingwood, L. E. (18. **VI**. 1898), A study of the growth of bacteria upon media made from animal organs. *Centralbl. f. Bakt.*, xxiii. pp. 982, 1002, 1043.

Ljubinsky. (Quoted by Blumenthal and Lipskerow.)

Lobstein, J. F. (1817), Observations et recherches sur le croup. *Mém. de la soc. méd. d'émulation*, viii. année (2), 500–568. Paris.

Loeffler, F. (1884), Untersuchungen über die Bedeutung der Mikroorganismen für die Enstehung der Diphtherie beim Menschen, bei der Taube und beim Kalbe. *Mitth. a. d. K. Gesundheitsamte*, ii. 451.——(1887), *Centralbl. f. Bakt.*, ii. 105. (Report of Meeting.)——(1887), *Vorlesungen über die geschichtliche Entwickelung der Lehre von den Bacterien.* Leipzig: Vogel 252 pp.——(1890), Welche Maasregeln erscheinen gegen die Verbreitung der Diphtherie geboten? *Berlin. klin. Wochenschr.*, xxvii. 885 (also ref. *Hygien. Rundsch.*, i. 13).——(1890), Der gegenwärtige Stand der Frage nach der Entstehung der Diphtherie. *Deutsche med. Wochenschr.*, xvi. 81, 108.——(29. **IV**. 1890), Bemerkungen zu der Arbeit von Prof. E. Klein "Zur Aetiologie der Diphtherie." *Centralbl. f. Bakt.*, vii. 528.——(1891), Zur Therapie der Diphtherie. *Deutsche med. Wochenschr.*, xvii. 353.——(18. **X**. 1894), Die lokale Behandlung der Rachendiphtherie. *Deutsche med. Wochenschr.*, No. 42.——(22. **XI**. 1894), Zur Diphtheriefrage. *Deutsche med. Wochenschr.*, xx. 881.——(19. **XII**. 1901), Hygiene der Molkereiprodukte. *Deutsche med. Wochenschr.*, pp. 889, 909.——(1907), Neue Verfahren zur Schnellfärbung von Mikroorganismen, insbesondere der Blutparasiten, Spirochäten, Gonococcen und Diphtheriebacillen. *Deutsche med. Wochenschr.*, p. 169.

Loir, A. and Ducloux, E. (**VIII**. 1894), Contribution à l'étude de la diphtérie aviaire en Tunisie. *Ann. de l'Inst. Pasteur*, viii. 599.

Lommel (1897), Pathological conditions in the middle ear and sphenoidal sinus in true diphtheria. *Arch. Otol.*, N. Y., xxvi. 150 (quoted by Councilman, Mallory, and Pearce, 4. xii. 1900).

Long (1905), Diphtheria, its history and control by antitoxin. *Ann. Gynec. a. Pediatr.*, Boston, xviii.

Longstaff (1889), Supplement on diarrhœa and diphtheria in continuation of the *Rep. Med. Officer Loc. Gov. Board for* 1887.——(1891), *Studies in Statistics.*

Longuet (12. **XI**. 1892), Les origines de la diphtérie. *La Semaine Méd.*, xii. 446.

Longyear (**X**. 1897), Puerperal diphtheria. *Amer. Journ. of Obstetr.* (Ref. *Med. Chronicle*, vii.)

Loos, J. (28. **V**. 1896), Untersuchungen über das Verhalten des Blutserums gesunder und diphtheriekranker Kinder zum Diphtherietoxin. *Wien. klin. Wochenschr.*, ix. 455.——(1896), Ueber das Verhalten des Blutserums und diphtheriekranker Kinder zum Diphtherietoxin. *Jahrb. f. Kinderheilk.*, xlii. 360.

Lovett, and Munro (1887), Results in 327 cases of Tracheotomy. *Amer. Journ. Med. Sci.*, xliv.

Lubenau, C. (12. **IX**. 1901), Hämolytische Fähigkeit einzelner pathogener Schizomyceten. *Centralbl. f. Bakt.*, xxx. 356.

Lublinski (1894), Ueber eine Nachwirkung des Antitoxins bei Diphtherie. *Deutsche med. Wochenschr.*, xx.

Lubowski, R. (1900), Ueber einen atoxischen und avirulenten Diphtheriestamm und über die Agglutination der Diphtheriebacillen. *Zeitschr. f. Hyg.*, xxxv. 87.

Lusini, V. (1897), Di un caso di difterite per contagio immediato in soggetto adulto. *La Rif. Med.*, No. 158. (Ref. *Centralbl. f. Bakt.*, xxv. 195.)

Macfadyen, A. (1. **II**. 1900), On the influence of the temperature of liquid air on bacteria. *Proc. Roy. Soc.*, lxvi. 180.

Macfadyen, A. and Hewlett, R. T. (1900), A diphtheria-like organism found in pigeons. *Trans. Path. Soc.*, li. 13.

Macfadyen, A. and Rowlands, S. (5. **IV**. 1900), Further note on the influence of the temperature of liquid air. *Proc. Roy. Soc.*, lxvi. 339, 488.

Macgregor, A. (1898), The vitality of the Bacillus diphtheriae. *Lancet*, i. 716.

MacKenzie, J. J. (10. I. 1900), A Streptothrix form isolated from water, resembling the diphtheria bacillus. *Journ. of Boston Soc. of Med. Sci.*, iv. 77.

Macy, W. A. (1900), Observations on an outbreak of diphtheria. *Bull. New York State Board of Health.* (Ref. *Boston Med. and Surg. Journ.*, cxliii. 274.)

Madsen, Th. (1896), *Experimentelle Undersögelser over Difterigiften*, Copenhagen. ——(1897), Zur Biologie des Diphtheriebacillus. *Zeitschr. f. Hyg.*, xxvi. 157. (1897), Ueber Messung der Stärke des antidiphtherischen Serums. *Zeitschr. f. Hygiene*, xxiv. 425.——(1899), Ueber Heilversuche im Reagensglas. *Ibid.* xxxii.——(**VII.** 1899), La constitution du poison diphtérique. *Ann. de l'Inst. Pasteur*, xiii. 568.——(**XI.** 1899), La constitution du poison diphtérique. *Ibid.* xiii. 801.——(1902), The Decrease of Antibodies in the Organism indicated by a Formula. *Fest-Skrift, Copenhagen*, viii. 11.——(1903), La constitution du poison diphtérique. *Centralbl. f. Bakt.*, I. Abt., xxxiv. 630.

Madsen, Th. and Warlburn, L. (1904), Toxines et antitoxines de la ricine et de l'antiricine. *Overs. o. d. k. Danske Vidensk. Selsk. Forh.*, Copenhagen, 81.

Mallory, F. B. (1901), Necroses of the liver. *Journ. Med. Research*, i. 264—280.

Malouin (1746–47), *Hist. et Mém. de l'Acad. Roy. d. Sci.*

Manfredi, L. (1886), Sulla presenza di batteri morti nei follicoli dell' intestino di coniglio. *Giorn. internaz. d. sci. med.*, fasc. iv. (Ref. *Baumgarten's Jahresber.*, ii. 376.)

Mann, A. (1858), *Die auf uns gekommenen Schriften des Kappadociers Aretaeus aus dem Griechischen übersetzt.* Leipzig.

Marcuse, J. (1875), Experimentelle Untersuchungen über die Erzeugung eines croupösen Processes auf der Trachealschleimhaut von Kaninchen. *Deutsche Zeitschr. f. Chir.*, v. 631.

Marcuse, P. (1899), Ist Diphtherie-Heilserum ein Heilmittel? *Arch. f. Kinderheilk.*, xxvi. 383.

Marenghi, G. (1897), Ueber die gegenseitige Wirkung des antidiphtherischen Serums und des Diphtherietoxins. *Centralbl. f. Bakt.*, xxii. 520.——(1897), Ueber die Beziehung zwischen der Ausscheidung des Stickstoffs in Stoffwechsel des Pferdes und der Erzeugung des Diphtherieserums. *Ibid.* xxi. 256.

Marf, J. (1899), Ein Beitrag zur Aetiologie der genuinen Rhinitis fibrinosa. *Korrespdzbl. f. Schweizer Aerzte*, p. 645. (Ref. *Baumgarten's Jahresber.*, xv. 257.)

Marmier, L. A. (1896), Les toxines et l'électricité. *Ann. Inst. Pasteur*, x. 469.

Marpmann (1899), Der Diphtheriebacillus und seine nächsten Verwandten. *Zeitschr. f. angew. Mikrosk.*, v. 135. (Ref. *Centralbl. f. Bakt.*, xxvii. 685.)

Marsden, R. W. (8. **IX.** 1900), Diphtheria and its treatment by Antitoxin. *Brit. Med. Journ.*, ii. 658.——(**VIII.** 1901), Post-scarlatinal diphtheria. *Med. Chronicle*, i. (Ser. iv.), 337.

Marshall, W. E. (1. I. 1907), *Journ. of Hygiene*, vii. p. 32. Note on the occurrence of diphtheria bacilli in milk.

Martha (1893), Note sur un cas de diphtérie attenuée. *Arch. de Méd. expér. et d'anat. path.*, v. 688. (Ref. *Baumgarten's Jahresber.*, ix. 173.)

Martin (1903), Propriétés du sérum antidiphtérique. *Compt. rend. Soc. Biol.*, lv.

Martin, A. (1902–3), Diphtheria in relation to domestic animals. *Publ. Health*, xv. 533.

Martin, C. J. (1898), The curative value of Calmette's antivenomous serum in the treatment of inoculations of the poisons of Australian snakes. *Brit. Med. Journ.*, ii. 1805.

Martin, C. J. and Cherry, T. (1898), The nature of the antagonism between toxin and antitoxin. *Proc. Roy. Soc.*, lxiii. 420. Also *Brit. Med. Journ.*, ii. 1120.

Martin, L. (**V. 1892**), Examen clinique et bactériologique de deux cents enfants, entrés au pavillon de la diphtérie à l'hôpital des enfants malades. *Ann. de l'Inst. Pasteur*, VI. 335.——(**I. 1898**), Production de la toxine diphtérique. *Ibid.* XII. 26.——(**1903**), Propriétés du sérum antidiphthéritique. *Compt. rend. Soc. de Biol.*, IV. 624.

Martin, S. (**1891-2**), Report on the chemical pathology of diphtheria and of infective endocarditis: with an account of diphtheritic palsy experimentally produced by the chemical poison of diphtheria. *Local Govern't. Board Report*, XXI., Appendix B, p. 147.——(**1892**), Chemical pathology of anthrax, diphtheria and ulcerative endocarditis. *Brit. Med. Journ.*, I. 641, 696.——(**1894**), Report on chemical pathology of diphtheria in relation to the symptoms of the disease. *Rep. of Med. Officer of the Local Gov't. Board*, 1892–93, Appendix B, XXII. p. 427.——(**24. VIII. 1895**), The treatment of diphtheria by antitoxic serum. *Brit. Med. Journ.*——(**1896**), On the mode of action of the Bacillus diphtheriae. *Congr. internat. d'hyg. et de démog.*, II. 981.

Martini, L. de (**1896**), Sul comportamento del siero antidifterico filtrato attraverso lo candele Chamberland. *Riforma Med.*, No. 266, p. 484, also appeared in German in *Centralbl. f. Bakt.*, XX. 796. (Ref. *Baumgarten's Jahresber.*, XII. 239, 240.)——(**30. I. 1897**), Zur Differenzierung der Diphtherie von den Pseudodiphtheriebacillen. *Centralbl. f. Bakt.*, XXI. 87.

Marx, E. (**1901**), Experimentelle Untersuchungen über die Beziehung zwischen dem Gehalt an Immunitätseinheiten und dem schützenden und heilenden Werth der Diphtherieheilsera. *Zeitschr. f. Hygiene*, XXXVIII. 372.——(**1902**), *Die experimentelle Diagnostik, Serumtherapie u. Prophylaxie der Infektionskrankheiten.* (Bibliothek von Coler, Bd. 2.) Herausgegeben von O. Schzerning, Bd. 11, Berlin, Hirschwald.——(**1904**), Die Bestimmung kleinster Mengen Diphtherieantitoxins. *Centralbl. f. Bakt.*, XXXVI. 141.

Marzinowsky, E. J. (**25. VII. 1900**), Ueber einige in den Krypten der Gaumenmandeln gefundene Bacillenarten. *Centralbl. f. Bakt.*, XXVIII. 39.

Mason, A. P. (**I. 1901**), The Swab method in rapid diagnosis of diphtheria. *Journ. of the Mass. Assoc. of Boards of Health*, X. 99.

Massachusetts Assoc. of Boards of Health (**VI. 1902**), Report on diphtheria bacilli in well-persons.

Massachusetts State Board of Health (**1896**), 28*th Annual Report* (Persistence of diphtheria bacilli), p. 692.——(**1897**), 29*th Annual Report*, p. 602.——(**1898**), 30*th Annual Report*, p. 740.——(**1899**), 31*st Annual Report*, p. 671.——(**1900**), 32*nd Annual Report*.——(**1901**), 33*rd Annual Report*.——(**1902**), 34*th Annual Report*.——(**1903**), 35*th Annual Report*.——(**1904**), 36*th Annual Report*.

Masterman, G. F. (**23. I. 1886**), A contribution to the etiology of diphtheria. *Brit. Med. Journ.*, I. 152.

Maunier and Bertherand (**X. 1898**), Étude clinique et bactériologique sur un cas d'angine aigue diphtéroide à leptothrix. *Arch. de méd. des Enfants.* (Ref. *Med. Chron.*, X. 66.)

Maurans, de (**1901**), Les oscillations de la mortalité par diphtérie, sont-elles sous la dépendance immédiate des méthodes thérapeutiques? *Semaine Méd.*

May, E. (**1899**), *On the pathology and treatment of diphtheria.* London.

Mayer, G. (**31. V. 1899**), Ueber das Wachstum von Mikroorganismen auf Speicheldrüsen und Mucin-Nährböden. *Centralbl. f. Bakt.*, XXV. 747, 815.

McClure, C. (**30. VI. 1898**), Ueber einen in der Milch gefundenen Bacillus. *Deutsche med. Wochenschr.*, XXIV. 414.

McCollom, J. H. (**1895**), The importance of bacteriological investigations in cases of diphtheria. *Boston Med. and Surg. Journ.*, CXXXII. 49.——(**XI. 1897**), Two cases of diphtheria of the penis. *Journ. of Boston Soc. of Med. Sci.*, II. 22.——(**1898**), A clinical study of 800 cases of Diphtheria at the South Department of the Boston City Hospital. *Med. and Surg. Reports Boston City Hosp.*, 9th Ser., p. 1.——(**1905**), The experience of nine years in the treatment of diphtheria with antitoxin. *Boston Med. and Surg. Journ.*, CLII.

McKee, S. H. (**I. 1906**), Organisms normally present in the conjunctiva. *Montreal Med. Journ.*, XXXV. 1.

McKenzie, D. (**1903**), Nasal diphtheria and infection. *Brit. Med. Journ.*, I. 911.

Méguin, P. (**1891**), Sur la diphtérie du pigeon. *Compt. rend. Soc. Biol.*, IX. 593.

Meikle, J. H. (**XII. 1906**), Persistence of bacilli in the throats of convalescent diphtheria patients: a clinical and bacteriological study of three hundred cases. *Edinburgh Med. Journ.*, XX. 510.

Menard (**1890**), Sobre la no identidad de la difteria humana y la gue padecen las ares. *An. r. Acad. de med.*, X. 150; also *Rev. d'hyg.*

Mensi (**1898**), La rinite difterica primitiva in rapporto alla profilassi della difterite. *Riv. d' Ig.*, p. 1. (Ref. *Baumgarten's Jahresbericht*, XIV. 296.)

Méry (**1895**), Persistance des bacilles dans la gorge après la diphtérie. *Bull. et mém. Soc. méd. d. hôp. de Par.*, XII. 128.

Metchnikoff, E. (**1894**), Recherches sur le choléra et les vibrions 3 and 4 Mem. *Ann. Inst. Pasteur*, VIII. 257.——(**1901**), *L'immunité dans les maladies infectieuses.* Paris: Masson et Cie.——(**1905**), *Immunity in Infective Diseases.* Cambridge: University Press, 591 pp.

Metchnikoff, Roux and Salimbeni (**1896**), Toxine et antitoxine cholérique. *Ann. Inst. Pasteur*, X. 257.

Métin (**IX. 1898**), Le bacille de la diphtérie pullule-t-il dans les organes? *Ann. de l'Inst. Pasteur*, XII. 596.

Meyer, C. (**1890**), *Deutsches Archiv f. klin. Med.*, LIX. 465. (Ref. *Amer. Journ. Med. Sci.*, CXV. 467.)

Meyer, E. (**1896**), Bakteriologische Befunde bei Rhinitis fibrinosa. *Arch. f. Laryngol. u. Rhinol.*, IV. 96, 249. (Ref. *Centralbl. f. Bakt.*, XX. 697.)

Meyer, F. (**1902**), Zur Bakteriologie der experimentellen Endocarditis. *Internat. Beitr. z. inn. Med.*, II. 443. (Ref. *Centr. f. Bakt.*, XXXII. 106.)

Meyerhof, M. (**1898**), Zur Morphologie des Diphtheriebacillus. *Arch. f. Hyg.*, XXXIII. 1.

Michel, G. (**24. IX. 1897**), Das Wachstum der Diphtheriebacillen auf verschiedenen Sera und Glycerinagar. *Centralbl. f. Bakt.*, XXII. 259.

Michelazzi, A. (**1904**), (Bacteriological study of cases thought to be Diphtheria). *Il Policlinico*, No. 9. (Rev. *Amer. Med.*, VIII. 392.)

Middledorpf and Goldman (**1891**), *Experimentelle und pathol.-anatomische Untersuchungen über Croup und Diphtherie.* Jena.

Middleton (**1756**), *Cases of the angina trachealis.* New York.

Migula, W. (**1900**), *System der Bakterien*, Pt. II.

Millar, J. (**1769**), *Observations on the asthma and hooping-cough.* London.

Miller, E. C. L. (**1905**), On the keeping of antidiphtheritic serum. *Centralbl. f. Bakt.*, Abt. I., XXXVIII. 233.

Miller, W. D. (**1892**), *Die Mikroorganismen der Mundhöhle.* Leipzig.

Millikin, D. (**1890**), Diphtheria of the genitalia. *Med. and Surg. Reporter*, Philad., LXII. 197.

Minnesota State Board of Health (**1899, 1900**), *Biennial Report*, pp. 497, 513.

Mollard, J. and Regnaud, Cl. (**II. 1897**), Lésions du myocarde dans l'intoxication aigue par la toxine diphtérique. *Ann. de l'Inst. Pasteur*, XI. 97.——(**10. VII. 1897**), Lésions chroniques expérimentales du myocarde consécutives à l'intoxication diphtérique. *Compt. rend. Soc. Biol.*, Ser. X., IV. 674.——(**17. VII. 1897**), Note sur l'histogénèse des scléroses du myocarde produits par l'intoxication diphtérique expérimentale. *Ibid.*, Ser. x., iv. 755.——(17. VIII. 1897), Athérome de l'aorte chez des animaux soumis à l'intoxication diphtérique. *Ibid.*, Ser. x., iv. 756.

Möller, J. (1902), Ueber Rhinitis fibrinosa s. crouposa s. pseudo-membranacea (Danish). *Hosp. Tidende*, p. 971. (Ref. *Baumgarten's Jahresber.*, xviii. 211.) ——(1902), Zwei Fälle von Rhinitis fibrinosa. *Zeitschr. f. Ohrenheilk.*, lvi. 353. (Ref. *Centralbl. f. Bakt.*, xxxii. 534.)

Moncel, T. du (5. VIII. 1893), Durée de la période contagieuse chez les diphtériques convalescents, d'après l'examen bactériologique. *Province méd.*, vii. 361. (Ref. *La Sem. Méd.*, xiii. 415.)

Montefusco, A. (1896), Del modo di comportarsi del bacillo delle difterite sulle sostanze alimentarie. *Ann. d' Ig. sper.*, vi. (Ref. *Centralbl. f. Bakt.*, xxxi. 352.)

Monti, A. and Veratti, E. (II. 1895), Diagnosi batteriologica della difterite. *Boll. Società d. med. di Pavia.* (Ref. *Baumgarten's Jahresber.*, xi. 257.)

Moore, V. A. (1895), Investigations concerning infectious diseases among poultry. *U.S. Dep't. of Agricult. Bur. of Animal Industry, Bull.* No. 8, p. 39.——(II. 1901), The control of diphtheria in small cities and country districts from a bacteriological standpoint. *New York State Journ. of Med.* (Ref. *Baumgarten's Jahresber.*, xvii. 170.)

Moos, S. (1887), Untersuchungen über Pilz-Invasion des Labyrinthes im Gefolge von Diphtherie. *Zeitschr. f. Ohrenheilk.* (Ref. *Baumgarten's Jahresber.*, iii. 252.) ——(1890), Histologische und bakteriologische Untersuchungen über Mittelohrerkrankungen bei den verschiedenen Formen der Diphtherie. *Ibid.* xx. (Ref. *Centralbl. f. Bakt.*, x. 19.)

Morax, V. (IV. 1895), La conjunctivité diphtérique, son traitement par le sérum antitoxique. *Ann. d'oculist.* (Ref. *Centralbl. f. Bakt.*, xviii. 88.)

Morax, V. and Elmassian, M. (III. 1898), Action de la toxine diphtérique sur la muqueuse. *Ann. de l'Inst. Pasteur*, xii. 210.

Morel, C. (1891), *Contribution à l'étude de la diphtérie* (*bactériologie et anatomie pathologique*). Paris.

Morgenroth, J. (1899), Ueber den Antikörper des Labenzymes. *Centralbl. f. Bakt.*, xxvi. 349.——(1904), Untersuchungen über die Bindung von Diphtherietoxin und Antitoxin, sowie über die Constitution des Diphtheriegiftes. *Berlin. klin. Wochenschr.*, xli. 526, also *Zeitschr. f. Hyg.*, xlviii. 177.——(1905), Untersuchungen über die Bindung von Diphtherietoxin u. Antitoxin, zugleich ein Beitrag zur Kenntnis der Konstitution des Diphtheriegiftes. *Zeitschr. f. Hyg.*, xlviii. 177.

Morison, B. G. (26. V. 1900), Pseudodiphtheria. *Lancet*, i. 1542. (Letter.)

Moritz, G. (1893), Zur Kenntniss der Conjunctivitis fibrinosa. *Beitr. z. Augenheilk.* by Deutschmann, p. 47. (Ref. *Baumgarten's Jahresber.*, ix. 191.)

Morrell, J. and Wolf, H. E. (29. XII. 1906), Cases of meningitis with the isolation of diphtheria bacillus from cerebro-spinal fluid. *Journ. Amer. Med. Assoc.*, xlvii. 2137.

Morse, J. L. (15. and 22. II. 1894), A bacteriological study of four hundred cases of inflammation of the throat, occurring in diphtheria and scarlet fever, with especial reference to pathogenesis. *Boston Med. and Surg. Journ.*——(1894), Bacteriology of diphtheria. *Bost. City Hosp. Med. and Surg. Reports*, v. 29–54. ——(1895), A clinical and experimental study of the leucocytosis of diphtheria. *Ibid.* vi. 190–210.——(1899), The blood in diphtheria. *Ibid.* x.

Mosler (1873), Ueber Collaps nach Diphtherie. *Arch. d. Heilk.*, xiv.

Mosny, E. (1891), Étude sur les lésions, les causes et la prophylaxie de la bronchopneumonie. *Rev. mens. d. mal. de l'enf.*, Paris, ix. 49, 118.

Mosotto (1864), *Gaz. med. della prov. Venez.*

Muir, R. and Ritchie, J. (1899), *Manual of Bacteriology.* 2nd Ed.

Müller (1902), Beitrag zur Statistik der Diphtheriemortalität. *Jahrb. f. Kinderheilk.*, LV.

Müller, A. W. K. (9. II. 1899), Ueber seltenere Localisation des Diphtheriebacillus auf Haut und Schleimhaut. *Deutsche med. Wochenschr.*, XXV. 91.

Müller, F. (1896), Untersuchungen über das Vorkommen von Diphtheriebacillen in der Mundhöhle von nicht diphtherischen Kindern innerhalb eines grossen Krankensaales. *Jahrb. f. Kinderheilk.*, XLIII. 54. (Also ref. *Centralbl. f. Bakt.* (1897), XXI. 159 (Kossel).)——**(1898)**, Ueber die Resistenz des Diphtherieheilserums gegenüber verschiedenen physikalischen und chemischen Einflüssen. *Centralbl. f. Bakt.*, XXIV. 8.——**(15. VII. 1899)**, Ueber reduzierende Eigenschaften von Bakterien. *Centralbl. f. Bakt.*, XXVI. 51.

Müller, R. (10. III. 1906), Diphtheriebacillenähnliche Stäbchen bei Anginen mit scharlachartigen Exanthem. *Centralbl. f. Bakt.*, XL. 613.——**(1906)**, Zur Aetiologie der Geflügeldiphtherie. *Ibid.*, I. Abt., XLI. 423.

Munn, W. P. (VI. 1897), True diphtheria of the penis; with a report of three cases. *Pittsburgh Med. Rev.*, XI. 43.

Murajeff, B. (1901), Ueber die Diphtherie der Vögel. *Arch. f. Vet.-Wiss.*, p. 605. (Ref. *Baumgarten's Jahresber.*, XVII. 199.)

Murawjeff (1898), Die diphtherischen Toxine und Antitoxine in ihrer Wechselwirkung auf das Nervensystem des Meerschweinchen. *Fortschr. der Med.*, XVII. 93.

Murillo, F. (1903), Ueber die Diphtherietoxinkurve. *Centralbl. f. Bakt.*, XXXV. 203.

Mya, G. (1897), Ueber die Pathogenese der diphtherischen Bronchopneumonie. *Wien. med. Bl.*, XX. 243, 259, 277, 297.

Naether (1900), Versuche über die Beseitigung der Diphtheriebacillen aus der Mundhöhle von Reconvalescenten. *Deutsche militärärztl. Zeitschr.*, p. 241. (Ref. *Centralbl. f. Bakt.*, XXIX. 465.)

Nakanishi, K. (26. V. 1900), Bacillus variabilis lymphae vaccinalis, ein neuer Konstant in Vaccinepusteln vorkommender Bacillus. *Centralbl. f. Bakt.*, XXVII. 640.——**(8. VIII. 1901)**, Ueber den Bau der Bakterien. *Ibid.* XXX. 153.

Nash, J. T. C. (1906), A case of faucial angina with false membrane (non-diphtheritic) simulating diphtheria. *Lancet*, II. 722.

Nassiloff (1870), Ueber die Diphtheritis. *Virch. Arch.*, L. 550–566.

Nauwelaers (1895), Contribution à l'étude bactériologique des angines à fausses membranes diphtériques et pseudo-diphtériques. *Ann. Soc. roy. d. sc. méd. et nat. de Brux.*, IV. 1.

Nedrigailoff, V. I. (13. VI. 1901), [On the biology and morphology of old diphtheria cultures.] *Bolnitch. Gaz. Botkina*, XII. 24. (Ref. *Philad. Med. Journ.*, VIII. 1081), also *Baumgarten's Jahresber.*, XVII. 171.)

Neisser, A. (1888), Versuche über die Sporenbildung bei Xerosebacillen, Streptokokken und Choleraspirillen. *Zeitschr. f. Hyg.*, IV. 165.

Neisser, E. (1891), Ein Fall von Hautdiphtherie. *Deutsche med. Wochenschr.*, XVII. 703

Neisser, E. and Kahnert (1900), Ueber eine Gruppe klinisch und ätiologisch zusammengehöriger Fälle von chronischer Erkrankung der oberen Luftwege. *Deutsche med. Wochenschr.*, XXVI. 525.

Neisser, M. (1897), Zur Differentialdiagnose des Diphtheriebacillus. *Zeitschr. f. Hyg.*, XXIV. 443.——**(1898)**, *Ueber Luftstaubinfektion, ein Beitrag zum Studium der Infektionswege.* Habilitationschrift, Leipzig. (Ref. *Centralbl. f. Bakt.*, XXIV. 704.)——**(1903)**, Die Untersuchung auf Diphtheriebacillen in centralisierten Untersuchungstationen. *Hyg. Rundsch.*, XIII. 705.——**(1904)**,

Enquête des ärztlichen Vereins zu Frankfurt a. M. über eine Diphtherieepidemie April-Mai 1903. *Berlin. klin. Wochenschr.*, xli. 283.——(1905), Zur Diagnostik des Diphtheriebazillus. *Deutsche Aerztl.-Ztg.*, 1.

Nencki, M., Sieber and Schoumow-Simanowski, E. (1898), Entgiftung der Toxine durch die Verdauungssäfte. *Centralbl. f. Bakt.*, xxiii. 840, 880.

Nernst, W. (1904), Ueber die Anwendbarkeit der Gesetze der Gemische von Toxin und Antitoxin. *Zeitschr. f. Electrochemie*, x. 377.

Netter (1892), Étude bactériologique de la broncho-pneumonia chez l'adulte et chez l'enfant. *Arch. de Méd. expér. et d'Anat. path.* iv. 28–65.——(1891), *Bull. et mém. de la Soc. Méd. des hôpit. de Paris*, viii. 224.——(1902), Des Injections préventives du sérum antidiphtérique dans les familles. *Presse méd.*, i. 387, 389.——(1906), Efficacité de l'ingestion de chlorure de calcium comme moyen préventif des éruptions consécutives aux injections de sérum. *Compt. rend. Soc. Biol.*, lx.

Neufeld, L. (1904), Ueber chronische Diphtherie. *Deutsche med. Wochenschr.*, xxx. 738.

Neumann, A. (1891), Aetiologie der Diphtherie. *Arch. f. Kinderheilk.*, xii. (Ref. *Baumgarten's Jahresber.*, vii. 235.)——(16. II. 1893), Mittheilungen über Diphtherie. *Deutsche med. Wochenschr.*, xix. 150.——(1898), *De l'influence de la tension gazeuse sur les microbes et en particulier sur le bacille de la diphtérie.* Toulouse (*Thesis*). (Ref. *Hyg. Rundsch.* ix. 1079.)

Neumann, E. (1896), Zur Kenntniss der fibrinoiden Degeneration des Bindegewebes bei Entzündungen. *Virch. Arch.*, cxliv. 201–238.

Neumann, R. O. (1902), Bakteriologische Untersuchungen gesunder und kranker Nasen, mit besonderer Berücksichtigung. des Pseudodiphtheriebacillus. *Zeitschr f. Hyg.*, lx. 33.——(1902), Virulente Diphtheriebacillen bei einfacher Rhinitis. *Centralbl. f. Bakt.*, xxxi. 33.

Newsholme, A. (9. and 16. III. 1895), Milroy Lectures on the natural history and affinities of Rheumatic Fever. *Lancet*, i.——(1898), *The Origin and Spread of Pandemic Diphtheria.* London.——(1904), Protracted and recrudescent infection in scarlet fever and diphtheria. *Med. Chir. Trans.*, lxxxvii. 549.——(9. III. 1904), The rôle of "Missed" cases in the spread of infectious diseases. *Public Health Lab. Victoria Univ. of Manchester.* 22 pp. repr.

Nicolas, J. (25. VI. 1896), Production de la réaction de Gruber et Durham par l'action du sérum anti-diphtérique sur le bacille de Loeffler. *Compt. rend. Soc. Biol.*, iii. 817.——(5. XII. 1896), Atténuation du bacille de Loeffler ayant subi la réaction agglutinante par l'action du sérum anti-diphtérique. *Ibid.* iii. 1025.——(4. VI. 1898), L'agglutination du bacillus de Loeffler par le sérum anti-diphtériques est-elle constante? *Ibid.*, Ser. x., v. 627.——(20. X. 1898), Sur la constance de l'aptitude ou de l'inaptitude de certains échantillons du bacille de Loeffler à se laisser agglutiner par divers sérums anti-diphtériques. *Ibid.* v. 1001.——(3. XII. 1898), Des rapports de l'agglutinabilité de divers échantillons de B. de Loeffler avec leur virulence et du pouvoir préventif du sérum anti-diphtérique à leur égard. *Ibid.* v. 1127.——(13. X. 1900), Note sur l'acquisition de l'agglutinabilité par un bacille de Loeffler primitivement non agglutinable. *Ibid.* vii. 837.

Nicolas, J. and Arloing, F. (1899), Influence de divers milieux nutritifs sur la végétabilité et la virulence du bacille de Loeffler. *Compt. rend. Soc. Biol.*, vi. 991.

Nicolas, J. and Cournont, P. (1898), A propos de la leucocytose dans la diphtérie. *Arch. de Méd. Expér.*, x. 592.

Nicolle (1895), Pratique des colorations microbiennes. *Ann. de l'Inst. Pasteur*, ix. 664.——(VI. 1896), Préparation de la toxine diphtérique. *Ibid.* x. 333.

Nicolle, C. and Herbert, M. A. (1897), Les angines à bacille Friedländer. *Ann. de l'Inst. Pasteur*, xi.

Nigris, B. de (1901), Sui metodi per la ricerca dei granuli polari nel bacillo della difterite. *Ann. d'Ig. sper.*, xi. 426. (Ref. *Baumgarten's Jahresber.*, xvii. 176.)

Nikanorow, P. J. (1897), Ueber die Gewinnung von Diphtherieheilserum von hohem Antitoxingehalt. *Berlin. klin. Wochenschr.*, xxxiv. 720.

Nisot (1896), *Bull. de la Soc. Belge de Gyn. et d'Obstét.*, iii. (Quoted by Salmon, 1904.)

Nissen, F. (1889), Zur Kenntniss der bacterienvernichtenden Eigenschaft des Blutes. *Zeitschr. f. Hygiene*, vi. 487.

Nobleville, Arnaud de (1748), *Histoire et mémoires de l'académie royale des sciences*, p. 324.

Nobécourt, P. (28. VII. 1900), Action des levures sur la virulence du bacille de Loeffler et sur la toxine diphtérique. *Compt. rend. Soc. Biol.*, lii. 753.

Novy, F. G. (13. VII. 1895), The etiology of diphtheria. *Med. News*, 23 pp. repr.

Nowak, J. (30. V. 1896), Blutbefunde bei an Diphtherie verstorbenen Kindern. *Centralbl. f. Bakt.*, xix. 982–991.

Nuttall, G. H. F. (1888), Experimente über die bacterienfeindlichen Einflüsse des thierischen Körpers. *Zeitschr. f. Hygiene*, iv. 353.

Oberwinter (1903), Ueber die nach Injection von Diphtherieheilserum auftretende Exantheme. *Deutsche med. Wochenschr.*, xxix.

Oertel, J. (1887), *Die Pathogenese der epidemischen Diphtherie nach ihrer histologischen Begründung.* Leipzig.

Oertel, M. (1888), Studien über Diphtherie. *Aerztl. Intell.-Bl.*, xv. 407.——(1871), *Experimentelle Untersuchungen über Diphtherie.* Leipzig.——(1871), *Deutsches Arch. f. klin. Med.*, viii. 242.

Ohlmacher, A. P. (1895), Two examples illustrating possible sources of error in the diphtheria culture test. *Journ. Amer. Med. Assoc.*, xxiv. 311.——(2. II. 1895), Some suggestions in bacteriological technique. *New York Med. Journ.*——(16. III. 1895), A detailed report of some experiments in the production of the diphtheria-antitoxin. *Med. News*, 19 pp. repr.——(27. IV. 1895), The diphtheria bacillus in a pneumonic lung. *New York Med. Journ*, 529.——(4. V. 1895), Some improvements in the technique of the diphtheria culture test. *New York Med. News*, lxvi. 491.——(I. 1902), Observations on the morphologic variations in certain pathogenic bacteria. *Journ. Med. Research*, vii. 128.

Oppenheimer, C. (1904), *Toxine und Antitoxine.* Jena: Fischer.

Orlowski, W. (1895), Ueber die antitoxischen Eigenschaften des Blutserums bei Kindern. *Deutsche med. Wochenschr.*, p. 400.

Orth, J. (1887), *Lehrb. d. Spec. path. Anatomie*, 371.

Ortmann (1889), [Sitzungsbericht des Vereins für wissenschaftl. Heilkunde zu Königsberg]. *Berl. klin. Wochenschr.*, No. 10, 218.

Ortmann, P. and Samter, E. O. (1890), Beitrag zur Lokalisation des Diplococcus pneumoniae (Fraenkel). *Virchow's Arch.*, cxx. 94.

Otto, Das Theobald Smith'sche Phänomen der Serum Ueberempfindlichkeit. *v. Leuthold Gedenkschrift*, vol. i.

Ouchinsky (1893), Recherches sur la nature des poisons de la diphtérie et du choléra. *Arch. de méd. expér. et d'Anat. pathol.*, v. 293. (Ref. *Baumgarten's Jahresber.*, ix. 173.)

Pakes, W. C. C. (27. I. 1900), The application of bacteriology to public health. *Brit. Med. Journ.*, i. 186.——(3. II. 1900), [Sore throats.] *Lancet*, i. 311. (Rep. of Meeting of the Soc. of Med. Officers of Health.)

Palmirski, W. and Orlowski, W. (23. III. 1895), Ueber die Indolreaktion in Diphtheriebouillonkulturen. *Centralbl. f. Bakt.*, xvii. 358.

Papkaw (1895), Zur Frage über die Veränderungen des Herzmuskels bei Diphtherie. *Wratsch.*

Papasotirin (**1900**), Notiz über den Einfluss des Petroleums auf den Diphtheriebacillus. *München. med. Wochenschr.*, 40. (Ref. *Centralbl. f. Bakt.*, xxx. 886.)

Park, W. H. (**30. VII. and 6. VIII. 1892**), Diphtheria and allied pseudomembranous inflammations. *N. Y. Med. Record.*——(**11. II. 1893**), Diphtheria and allied pseudomembranous inflammations. *Ibid.*——(**25. I. 1896**), The present status of the use of bacterial cultures for the diagnosis of suspected diphtheria. *New York Med. Journ.*, lxviii. 107.——(**1896**), Mixed infection and the virulence of diphtheria bacilli. *Proc. New York Patholog. Soc.*, p. 79.——(**1900**), Bacteriology in Medicine and Surgery. London: Henry Kimpton, pp. 335–376.——(**1900**), Treatment of diphtheria and the antitoxic effects of antitoxic sera. *Pediatrics*, New York, xii.

Park, W. H. and Atkinson, J. P. (**1898**), The relation of the toxicity of diphtheria toxin to its neutralizing value upon antitoxin at different stages in the growth of culture. *Journ. Exp. Med.*, iii. 513.

Park, W. H. and Beebe, A. L. (**1894**), Diphtheria and pseudo-diphtheria; a report to Hermann. M. Biggs......on the bacteriological examination of 5,611 cases of suspected diphtheria, with the results of other investigations on the diphtheria and pseudo-diphtheria bacillus. *N. Y. Med. Record*, xlvi. 385.

Park, W. H. and Williams, A. W. (**1896**), The Production of Diphtheria Toxin. *Journ. of Exper. Med.*, i. 164.

Parkes, L. (**1897**), Some observations on the infectivity of diphtheria, and on the relation of diphtheria prevalence to school closure. *Trans. Epidemiol. Soc.*, xvi. 240.——(**1902–3**), Prevention of diphtheria outbreaks in hospitals for children. *Publ. Health*, xv. 538.——(**11. IV. 1903**), *Brit. Med. Journ.*, i. 853. (Report of meeting of Epidemiolog. Soc. on 20. III. 1903.)

Parsons, H. F. (**1900**), On the comparative mortality of English Districts. *Trans. Epidemiol. Soc.*, xix. 10.

Passini, F. (**1896**), Versuche über die Dauer der antidiphtherischen Schutzimpfung. *Wien. klin. Wochenschr.*, ix. 1111. (Ref. *Baumgarten's Jahresber.*, xii. 241.)

Pasteur, W. (**27. V. 1905**), On pneumococcal sore throat with notes of a fatal case. *Lancet*, i. 1409.

Paterson, A. R. (**1866**), Case of diphtheria by inoculation appearing on a wound without the corresponding affection of the fauces, and followed by paralysis. *Med. Times and Gaz.*, ii. 608.

Paton, Dunlop and Macadam (**1899**), On the modifications of the metabolism produced by the administration of diphtheria toxin. *Journ. of Physiol.*, xxiv. 331.

Pauli, Fr. (**1865**), *Der Croup*, 2nd ed. Würzburg. [Quotes Duval, Schloepfer, Valentin.]

Paulinis (**22. I. 1888**), [The etiology of an epidemic of diphtheria.] *Bulletin Méd.* (Rev. in *Journ. of Comp. Pathol. a. Therap.*, i. 159.)

Paulsen (**5. IX. 1890**), Mikroorganismen in der gesunden Nasenhöhle und beim akuten Schnupfen. *Centralbl. f. Bakt.*, viii. 344.

Pearce, R. M. (**V. 1898**), The general infections and complications of diphtheria and scarlet fever. A bacteriological study of one hundred and fifty-seven cases. *Med. and Surg. Reports Boston City Hosp.*, ix. 86–105; also *Journ. Boston Soc. Med. Sci.*, ii. 92.——(**1899**), The bacteriology of the Accessory Sinuses of the nose in diphtheria and scarlet fever. *Journ. Bost. Soc. Med. Sci.*, iii. 215–223.

Peck, H. (**4. V. 1895**), The transmission of diphtheria by non-sufferers. *Brit. Med. Journ.*, i. 971.——(**16. II. 1901**), *Lancet*, i. 480. (Meeting of the Sheffield Medico-Chirurg. Soc.)

Peck, J. W. (**10. I. 1903**), A new differential stain for the Klebs-Loeffler bacillus of diphtheria. *Lancet*, i. 92.

Pegler, L. H. (**16. IX. 1905**), *Brit. Med. Journ.* ii. 651.

Pennington, M. E. (**1907**), The virulence of diphtheria organisms in the throats of well school-children and diphtheria convalescents. *Journ. of Infect. Diseases*, iv. 36.

Penzoldt (**1887**), Einige Versuche Diphtherie auf Thiere zu übertragen. *Deutsches Arch. f. klin. Med.*, xlii. 193. (*Baumgarten's Jahresber.*, iii. 246.)

Perkins, R. G. (**V. 1901**), Bacteriology and pathology of diphtheria. *Cleveland Med. Gaz.* (Ref. *Baumgarten's Jahresber.*, xvii. 171.)

Pernice, B. and Scagliosi, G. (**1895a**), Sulle alterazioni istologiche e sulla vitalità dei bacilli di Loeffler nelle pseudomembrane difteriche dell' uomo, studiate fuori l' organismo. *La Rif. Med.*, 1895, pp. 142–144. (Ref. *Centralbl. f. Bakt.*, xix. 723.)——(**1895b**), Histologische Untersuchungen des Nervensystems bei Diphtherie. *Ibid.* pp. 231–233. (Ref. *Centralbl. f. Bakt.*, xix. 887.)

Pes, O. (**23. V. 1896**), Ueber die Aetiologie und Therapie einiger Formen von Conjunctivitis pseudomembranosa. *Arch. f. Augenheilk.*, xxxii. 33. (Ref. *Centralbl. f. Bakt.*, xix. 729.)——(**1899**), Note batteriologiche sul bacillo del sebo meibomiano (Reymond-Colomiatti) nelle affezioni congiuntivali e sulle sua affinità biologiche col bacillo di Loeffler. *Riforma Med.*, p. 63. (Ref. *Baumgarten's Jahresber.*, xv. 217.)——(**1902**), Klinische und bakteriologische Beobachtungen über den akuten Bindehautkatarrh. *Arch. f. Augenheilk.*, xlv. 205. (Ref. *Centralbl. f. Bakt.*, xxxii. 269.)

Peter (**1859**), *Recherches sur la diphthérie.* Thèse, Paris.

Peter, L. C. (**14. II. 1903**), Post-diphtheritic paralysis affecting the general nervous system. *Med. News*, lxxxii. 296.

Peters. [Indol.] (Quoted by Flügge.) *Die Mikroorganismen*, vol. ii. p. 462.

Peters, A. (**25, II. 1897**), Ueber das Verhältniss der Xerosebacillen zu den Diphtheriebacillen, nebst Bemerkungen über die Conjunctivitis crouposa. *Deutsche med. Wochenschr.*, xxxiii. 133.

Peters, E. A. (**1896**), Diphtheria and pseudo-diphtheria bacilli. *Trans. Path. Soc.*, xlvii. 345.——(**1897**), Diphtheria and pseudo-diphtheria bacilli. *Journ. Path. a. Bact.*, iv. 181.

Peters, R. (**1882**), Ueber die hyaline Entartung bei der Diphtheritis des Respirationstractus. *Virch. Arch.*, lxxxvii. 477–509.

Petri (**1890**), *Arb. a. d. Kaiserl. Gesundheitsamte*, vi. i. (Quoted by Hewlett, 1900.)

Petrie, G. F. (**7. IV. 1905**), On the relationship of the pseudodiphtheria to the diphtheria bacillus. *Journ. of Hyg.*, v. 134.

Pfaffenholz (**15. VIII. 1895**), Zur bacteriologischen Diphtheriediagnose. *Hyg. Rundsch.*, v. 733.

Pfeiffer, H. (**25. VI. 1903**), Ueber Bakterienbefunde in der normalen männlichen Urethra und den "Syphilisbacillus" Max Josephs. *Wien. klin. Wochenschr.*, xvi. 762.

Pfeiffer, L. (**1888**), Beiträge zur Kenntniss der pathogenen Gregarinen. *Zeitschr. f. Hyg.*, v. 363.

Pfeiffer, R. and Friedberger, E. (**1902**), Ueber Antikörper gegen die bacteriolytischen Immunkörper der Cholera. *Berlin. klin. Wochenschr.*, xxxix. 4.

Philpot (**28. II. 1891**), Milk diphtheria. (A Report by the Croydon Medical Officer of Health.) *Brit. Med. Journ.*, i. 476.

Piazza, G. (**1895**), Sull' influenza della luce solare sulla tossina difterica. *Ann. d' Igiene sper.*, v. 521. (Ref. *Baumgarten's Jahresber.*, xi. 206.)

Pick (**1902**), *Zur Kenntnis der Immunkörper.* Brunswick : Vieweg and Sohn, p. 352.

Pick, E. P. and Schwoner, J. (**1904**), Beiträge zur Kenntnis des Diphtherieantitoxins und seiner Beziehungen zum Toxins. *Wien. klin. Wochenschr.*, xvii. 1055.——(**1905**), Untersuchungen über Diphtherieantitoxin und dessen Beziehungen zum Toxin. *Zeitschr. f. exper. Path. u. Ther.*, i. 98.

Pilcher (1896), Zur Frage der diphtheritischen Bindehautentzündung. *Beitr. z. Augenheilk.*, p. i. (Ref. *Centralbl. f. Bakt.*, xxi. 24.)

Piltz (1890), *Ein Beitrag zur Kenntniss der infantilen Xerosis Conjunctivae.* Halle. (Quoted by Stephenson, 1898.)

Pinckard, C. P. (1899), Diphtheritic conjunctivitis. *Journ. Amer. Med. Assoc.*, xxxii. 1135.

Piorkowski (19. XII. 1900), [Modification of stain for diphtheria bacilli.] Orig. Ref. *Centralbl. f. Bakt.*, xxix. 63.——**(4. III. 1901)**, Beitrag zur Färbung der Diphtheriebacterien. *Berlin. klin. Wochenschr.*, xxxviii. 236.

Pirquet, v. and Schick (1905), *Die Serumkrankheit.* Leipzig.

Pispoli, R. (1897), Sedici casi di difterite delle ferite. *Giorn. Med.*, No. 10. (Ref. *Centralbl. f. innere Med.*, xix. 1097.)

Pitfield, R. L. (1886), The bacteriological study of the etiology and diagnosis of diphtheria. *Pub. Health*, Philad., i. 108.——**(1897)**, Bacteriologic examinations in diphtheria, with a report of 160 cases. *University Med. Mag.*, ix. 767. (Ref. *Baumgarten's Jahresber.*, xiii. 256.)——**(IX. 1901)**, I. On the diagnosis of diphtheria. II. A double stain for the Bacillus diphtheriae. *Univ. of Pennsylvania Med. Bull.* (Ref. *Baumgarten's Jahresber.*, xvii. 177.)

Pitts, B. (3. IV. 1897), Diphtheria of the umbilicus. *Lancet*, i. 953.

Plaut, H. C. (6. XII. 1894), Studien zur bacteriellen Diagnostik der Diphtherie und der Anginen. *Deutsche med. Wochenschr.*, xx. 920.——**(2. V. 1895)**, Werth des Ausstrichpräparats bei der Diagnose der Diphtherie. *Ibid.* xxi. 291.

Plimmer, H. G. (1897), The bacteriological diagnosis of diphtheria. *St Mary's Hosp. Gaz.*, ii. 121.

Pluder, F. (29. X. 1896), Ueber Rhinitis fibrinosa diphtheritica. *Deutsche med. Wochenschr.*, xxii. 708.

Podack (1896), Ueber die Beziehungen des sogenannten Maserncroups und der im Gefolge von Diphtherie auftretenden Erkrankungen des Mittelohres zum Klebs-Loeffler'schen Diphtherie-bacillus. *Deutsch. Arch. f. klin. Med.*, lvi. 34.

Polano (1904), Antitoxinübergang von der Mutter auf das Kind. *Hyg. Rundschau*, xiv. 1245.

Pollock, W. B. I. (1905), The bacteriology of Conjunctivitis. *Trans. Ophthal. Soc.*, xxv. 3.

Post, A. (X. 1897), Diphtheria of the prepuce. *Boston Journ. Med. Sci.*, ii. 6.

Potter, T. (1899), Diphtheria and membranous croup. *Philadelphia Monthly Med. Journ.*, i. 417.

Power, W. H. (1885–6), Milk-Scarlatina in London in 1885. *Local Govern't. Board Report*, xv. 73, Append. A.

Poynton, F. J. (1900), A study of the heart wall in diphtheria, etc. *Lancet*, i. 1352.

Pratt, J. H. (1904), On the causes of cardiac insufficiency. *Johns Hopkins Hosp. Bull.*, xv. 301–309.

Preisich (1899), Zur Bakteriologie der Diphtherie und über Mischinfektion. *Jahrb. f. Kinderheilk. u. phys. Erzieh.*, xlviii. 271. (Ref. *Centralbl. f. Bakt.*, xxv. 193.)

Preisz, H. (1894), Beitrag zur Anatomie der diph. Lähmung. *Zeitschr. f. Nervenheilk.*, vi.——**(1893)**, Diphtheritis es pseudodiphtheritisbacillusok. (Hungarian.) *Orvosi Hetilap.*, No. 8. (Ref. *Baumgarten's Jahresber.*, ix. 191.)——**(1895)**, [The pathological anatomy of diphtheritic paralysis]. *Deutsche Zeitschr. f. Nervenheilk.*, No. 6. (Rev. in *Amer. Journ. Med. Sci.*, cx. 98.)

Prescott, W. H. (I. 1898), Diphtheria of the skin of the neck. *Journ. Boston Soc. Med. Sci.*, ii. 48.

Priestly, J. (**1902–3**), History of an outbreak of pseudo-diphtheria. *Pub. Health*, xv. 567.——(**II. 1906**), An outbreak of diphtheria and Vincent's angina. *Ibid.* xviii. 306.——(**1906**), History of a diphtheria outbreak (complicated with Vincent's angina) at the Lambeth Parochial Schools, West Norwood, May to July 1905. *Practitioner*, lxxvii. 372.

Prip, H. (**1900**), Studier over Blandingsinfection red Difteri. [Studies on mixed infection in Diphtheria.] *Dissert. Copenhagen.* (Ref. *Baumgarten's Jahresber.*, xvi. 194.)——(**1901**), Ueber Diphtheriebacillen bei Reconvalescenten nach Diphtherie. *Zeitschr. f. Hyg.*, xxxvi. 283.

Prochaska, A. (**1896**), Die Pseudodiphtheriebacillen des Rachens. *Zeitschr. f. Hyg.*, xxiv. 373.

Pröscher (**1902**), Ueber eiweissfreies Diphtherieantitoxin. *München. med. Wochenschr.*, xlix. 1176.—Patentanmeldung, F. 13, 756. 20. VI. 02.

Proskauer and Wassermann (**1892**), Ueber die von den Diphtheriebacillen erzeugten Toxalbumine. *Deutsche med. Wochenschr.*, No. 17.

Prudden, T. M. (**IV. 1889**), On the Etiology of Diphtheria. *Amer. Journ. Med. Sci.*, xcvii. 329.——(**18. IV. 1891**), Studies on the Etiology of Diphtheria. *Medical Record*, p. 445; also reprinted in *Studies from the Path. Lab. of the Coll. of Phys. and Surg's. Colombia Univ. N. Y.* 1890–1.

Prudden, T. M. and Northrup, W. P. (**1889**), Studies on the etiology of the pneumonia complicating diphtheria in children. *Amer. Journ. Med. Sci.*, xcvii. 562.

Pugh, W. T. G. (**VII. 1902**), Post-Scarlatinal Diphtheria. *Journ. Hyg.*, vol. ii. 286. ——(**8. VII. 1905**), A note on the examination of cultures and smears from the throat and nose. *Lancet*, ii. 80.

Pütz, H. (**1887**), Ueber croupös diphtheritische Erkrankungen unserer Hausthiere und deren Beziehungen zur Diphtherie des Menschen. *Oesterr. Zeitschr. f. wissensch. Veterinärk.*, i. 23.

Rahn (**1906**), Die Diphtherie-Serumtherapie und ihre Statistik. *Therap. Monatshefte*, xx.

Ramsey (**about 1786**), An account of the croup as it appeared in the town and neighbourhood of Chesham in Buckinghamshire. *Med. Transact. Roy. Coll. Phys. Lond.*, ii. 25.

Ranke, H. v. (**1896**), Zur Scharlachdiphtherie. *München. med. Wochenschr.*, No. 42.

Ransom, F. (**1900**), Diphtheritic paralysis and antitoxine. *Journ. of Pathol. and Bacteriol.*, vi. 397.

Ransom, W. B. (**1898**), Das Schicksal des Tetanusgiftes nach seiner Einverleibung in den Meerschweinchenorganismus. *Deutsche med. Wochenschr.*, xxiv. 117.

Raskin, M. (**28. and 29. III. 1889**), Klinisch-experimentelle Untersuchungen über Secundärinfection bei Scharlach. *Centralbl. f. Bakt.*, v. 433, 465.

Ratcliffe, J. N. (**1863**), On the recent epidemic of Diphtheria. *Trans. Epidem. Soc.*, i. 328. (Paper read 3. II. 1862.)

Raudnitz (**1902**), Zur bakteriologischen Diagnose der Diphtherie. *Prag. med. Wochenschr.*, p. 173.

Rautenberg (**1903**), [Discussion on Diphtheria in Königsberg.] *Deutsche med. Wochenschr.*, Vereinsbeilage, xxix.

Ravenel, M. P. (**18 and 25. V. 1895**), A contribution to the study of the etiology of membranous rhinitis. *Med. News*, 27 pp. repr.——(**10. VI. 1899**), The resistance of bacteria to cold. *Ibid.*

Real, J. de Villa (**1611**), *De signis, causis, essentia, prognostice et curatione morbi suffocantis.* Compluti.

Rebel, J. M. (**1890**), *Over de spontane verzwakkung der virulentie van den diphtheriebacil* (*Klebs-Loeffler*). Utrecht.

Recklinghausen, von (1871), *Centralbl. f. d. med. Wissensch.*, p. 713.

Rehns, J. (**9. II. 1901**), L'immunité active et les toxines diphtériques surcompensées. *Compt. rend. Soc. de Biol.*, 1901, p. 141. Republished in Ehrlich 1904, pp. 213—216.

Reiche, F. (**1895**), Nierenveränderungen bei Diphtherie. *Centralbl. f. innere Med.*, xvi. 1209-12.——(**1895**), Mischinfektion bei Diphtherie. *Centralbl. f. innere Med.*, xvi. 65.

Reichenbach, H. (**1899**), Ein Fall von Rhinitis fibrinosa mit Diphtheriebacillen. *Zeitschr. f. klin. Med.*, xxxviii. 486. (Ref. *Centralbl. f. Bakt.*, xxvii. 279.)

Remlinger, P. (**1899**), Contribution expérimentale à l'étude de la transmission héréditaire de l'immunité contre le bacille d'Eberth et du pouvoir agglutinant. *Ann. Inst. Pasteur*, xiii. 129.

Renny, H. (**VIII. 1905**), Diphtheria. *Public Health*, xvii. 714.

Répin (**1895**), Sur l'absorption de l'abrine par les muqueuses. *Ann. Inst. Pasteur*, ix. 517.

Report (**1879**) of the Committee on membranous croup and diphtheria. *Royal Med. a. Surg. Soc.*, London.

Reque, H. A. (**1906**), Some observations on phagocytosis of diphtheria bacilli. *Journ. Infect. Dis.*, iii. 440.

Reuter, Die Ergebnisse der Diphtheriebehandlung mit Behring'schen Heilserum in den Jahren 1898–1900. Mitteil. a. d. Hamburg Staatskrankenanstalten, 16 pp. 8⁰, Leopold Voss, Hamburg.

Reyes, A. (**1895**), Sulla propagazione del bacillo difterico per mezzo dell' aria. *Ann. d' Ig. sper.*, iv. (Ref. *Baumgarten's Jahresber.*, xi. 203.)

Reyes, C. (**1895**), Sulla vitalità del bacillo della difterite fuori dell' organismo e sulla possibile propagazione di esso attraverso l' aria. *Ann. d'Ig. sper.*, N.S., v. 501. (Ref. *Centralbl. f. Bakt.*, xix. 886.)

Ribbert, H. (**1885**), Ueber das Vorkommen von Spaltpilzen in der normalen Darmwand des Kaninchens. *Deutsche med. Wochenschr.*, xi. 197.——(**1896**), *Lehrb. d. patholog. Anat.*

Richardière and Tollemer (**1899**), Bacille pseudo-diphtérique et bacille diphtérique. *Presse Méd.* (Ref. *Centralbl. f. innere Med.*, xx. 809.)

Richardière, Tollemer and Ullmann (**1898**), Nouveaux faits de présence du bacille diphtérique dans le sang et dans les centres nerveux bulbo-protubérantices. *Bull. de mém. Soc. méd. de l'Hôp. de Par.*, xv. 22.

Richards, M. (**26. IX. 1896**), Post-scarlatinal diphtheria. *Lancet*, ii. 876.

Richmond, B. A. and Salter, A. (**1896**), The aetiological significance of the diphtheria bacillus and its variants. *Guy's Hospital Reports*, liii. 56.

Rickards, B. R. (**XI. 1906**), The value of consecutive negative cultures as a method of releasing diphtheria patients. *Amer. Journ. of Public Hygiene*, xvi. 25.

Ricker (**1895**), Ueber einen bemerkenswerten Fall von Streptokokkendiphtherie und über die intrauterine Infektion des Fötus mit dem Streptokokkus in diesem und in einem zweiten Falle. *Centralbl. f. allg. Pathol. u. pathol. Anat.*, vi. (Ref. *Centralbl. f. Bakt.*, xvii. 418.)

Riegler, E. (**1899**), Ueber die Behandlung der Rachendiphtherie mit Jodsäure und Wasserstoffsuperoxyd. *Wien. med. Blätter*, p. 845. (Ref. *Centralbl. f. Bakt.*, xxvii. 697.)

Riesman, D. (**5. III. 1898**), Two cases of diphtheria, one from laboratory infection and one in an infant eleven days old. *Philadel. Med. Journ.*, No. 10.

Rigler, G. von (**17. V. 1893**), Disinfektion mittelst Ammoniakdämpfen. *Centralbl. f. Bakt.*, xiii. 651.

Rindfleisch (**1886**), *Lehrb. d. patholog. Gewebelehre.*

Rist, E. (11. VII. 1903), Sur la toxicité des corps de bacilles diphtériques. *Compt. rend. Soc. de Biol.*, LV. 978.

Ritchie, J. (1901), Artificial modification of toxines with special reference to Immunity. *Journ. of Hygiene*, I. 125.

Ritter, J. (1894), Croup und Diphtherie. *Berlin. Klinik*, Hft. 73. (Ref. *Centralbl. f. Bakt.* XVI. 523.)——(1895), Thierdiphtherie und ansteckende Halsbräune. *Allg. med. Centralztg.*, No. 83 and 84. (Ref. *Baumgarten's Jahresber.*, XI. 271.)

Rittershain, v. (1902), Erfahrungen über Serum Exantheme. *Jahrb. f. Kinderheilk.*, LV.

Robert, J. (1895), Étude bactériologique de la diphtérie. *Arch. de méd. nav.*, LXIII. 44.

Robertson, W. (I. 1905), An outbreak of diphtheria traceable to ulcers on cow's teats. *Pub. Health*, XVII. 246.

Robertson, W. F. (VII. 1903), Histological evidence of the presence of an organism resembling the Klebs-Loeffler bacillus in cases of general paralysis of the insane. *Rev. of Neurol. and Psych.*, I. 470.——(24. X. 1903), The pathology of general paralysis of the insane. *Brit. Med. Journ.*, II. 1065.

Robertson, W. F. and McRae, G. D. (V. 1905), On the presence of diphtheroid bacilli in the genito-urinary tract in cases of general paralysis and tabes dorsalis. *Rev. of Neurol. and Psych.*, III. 321.

Robertson, W. F., McRae, G. D. and Jeffrey, J. (IV. 1903), Bacteriological investigations into the pathology of general paralysis of the insane. *Rev. of Neurol. and Psych.*, I. 225.——(V. 1903), Bacteriological investigations into the pathology of general paralysis of the insane. *Ibid.* I. 305.

Robertson, W. F. and Shennan, T. (IV. 1903), Experimental observations upon the pathogenic action of an organism resembling the Klebs-Loeffler bacillus, isolated from cases of general paralysis of the insane. *Rev. of Neurol. and Psych.*, I. 227.

Robin, A. (11. VIII. 1900), The etiology of diphtheria and the value of antitoxin. *New York Med. Journ.*, LXXII. 227.

Robinson, W. D. (16. V. 1896), Some experiences with the bacteriological department of the Philadelphia Board of Health. *Med. News*, LXVIII. 551.

Roeth, A. G. (1896), Micrococcus Brisou; a contribution to the study of diphtheria. *Boston Med. and Surg. Journ.*, CXXXIV. 83.

Roger et Bayeux (1887), Sur le rôle de la toxine diphtérique dans la formation des fausses membranes. *Compt. rend.*

Roger et Josue (1887), Action de la toxine et de l'antitoxine diphtérique sur la moelle osseuse. *Compt. rend.*

Rohmer (1903), Diphtherie-Statistik. *Arch. f. öffentl. Gesundh.*, XXII.

Rolleston (1905), Some aspects of the Serum treatment of Diphtheria. *Practitioner*, London, N.S., XXI.

Rolleston, J. D. (16. VI. 1906), A case of intranasal chancre simulating nasal diphtheria. *Lancet*, I. 1682.

Rolly, F. (1899), Ueber die Wirkung des Diphtheriegiftes auf das Herz. *Arch. f. exper. Pathol. u. Pharm.*, XLII. 283.

Romberg, E. (1891), Ueber die Erkrankungen des Herzmuskels bei Typhus abdominalis, Scharlach und Diphtherie. *Deutsch. Arch. f. klin. Med.*, XLVIII. 369.

Romberg and Passler (1899), *Deutsch. Arch. f. Klin. Med.*, LXIV. 652.

Römer, P. (1901), Der gegenwärtige Stand der Immunitätforschung. *Deutsche med. Wochenschr.*, XXVII. 529.

Roosen-Runge (21. VII. 1903), Ein Fall von Diphtheriebazillensepsis. *Münch. med. Wochenschr.*, L. 1252.

Rosenau, M. J. (1905), *The immunity unit for standardising diphtheria antitoxin (based on Ehrlich's normal serum).* Washington, Gov't. Print. Office, 92 pp.

Rosenau and Anderson (1906), A study of the cause of sudden death following the injection of horse serum. *Bull. No.* 29. *Hyg. Lab. U.S. Pub. Health and Marine Hosp. Service*, Washington, D.C., pp. 95.

Rosenbach (1902), Die Wellenbewegung der Seuchen und das Diphtherieserum. *Therap. Monatsh.*, xvi.

Rosenbach, J. (1877), Ueber Myocarditis diphtheritica. *Virch. Arch.*, lxx. 352.

Rosenberger, R. C. (23. VII. 1904), Two cases of Vincent's angina, and one of Noma in which the organisms characteristic of the former affection were found. *Amer. Med.*, viii. 161.

Rosenstein, Nil Rosen von (1798), *Anweisung zur Kenntnis und Kur der Kinderkrankheiten.* Aus dem schwedischen mit Zusätzen von J. A. Murray. 6. Auflage, pp. 354–384, Göttingen.

Roth (1883), *Adams Wochenschr.*, p. 173.

Roth, K. (1894), Zur bacteriologischen und klinischen Diagnose und Therapie der Diphtherie. *Jahrb. f. Kinderheilk.*, xxxviii. 96. (Ref. *Baumgarten's Jahresber.*, x. 227.)

Roux, E. (1900), Mésure de l'activité des sérums. *Congress Internat. d'Hygiène et Démographie*, 5.

Roux, E. and Borrel, A. (1898), Tétanos cérébral et immunité contre le tétanos. *Ann. Inst. Pasteur*, xii. 225.

Roux, E. and Martin, L. (1894), Contribution à l'étude de la diphtérie (Sérum-thérapie). *Ann. de l'Inst. Past.*, viii. 609.

Roux, E., Martin, L. and Chaillou, A. (1894), Trois cents cas de diphtérie traités par le sérum antidiphtérique. *Ann. de l'Inst. Past.*, viii. 640.

Roux, E. and Vaillard, L. (1893), Contribution à l'étude du tétanos. *Ann. Inst. Pasteur*, vii. 64.

Roux, E. and Yersin, A. (XII. 1888), Contribution à l'étude de la diphtérie. *Ann. de l'Inst. Past.*, ii. 629.——(1889), Contribution à l'étude de la diphtérie. *Ibid.* iii. 273.——(1890), Contribution à l'étude de la diphtérie. *Ibid.* iv. 385.

Rovaart, H. van de (23. IV. 1901), Zur Neisser'schen Färbung der Diphtheriebacillen. *Centralbl. f. Bakt.*, xxix. 574.

Royer-Collard, H. (1812), *Rapport sur les ouvrages du concours.* Paris.

Royston, L. T. (1. III. 1902), Diphtheria; with special reference to the symptoms and treatment. *Med. News*, lxxx. 386.

Rubens (24. XI. 1904), Zur Diagnose der Diphtherie. *Deutsche med. Wochenschr.*, xxx. 1771.

Rubinstein, S. (1903), Ueber das Verhalten einiger pathogener Bakterien in der Buttermilch. *Arch. f. Kinderheilk.*, xxxv. (Ref. *Centralbl. f. Bakt.*, xxxiv. 375.)

Ruediger, E. H. (1903), The occurrence of virulent pseudo-diphtheria bacilli in gangrenous tonsillitis and pharyngitis of scarlet fever. *Trans. Chicago Path. Soc.*, iv. 45.

Ruete (16. II. 1897), Ueber Herstellung des Diphtherieheilserums und seine Werthbemessung. *München. med. Wochenschr.*, p. 213.

Ruffer, M. A. (26. VII. 1890), Preliminary note on the processes taking place in diphtheritic membrane. *Brit. Med. Journ.*, ii. 202.

Runge, E. C. (1895), Inoculation into animals as the only decisive method for the biological diagnosis of true diphtheria; illustrated by three cases. *N. Y. Med. Journ.*, lxi. 586.

Rupp, A. (9. XI. 1901), Concerning the clinical significance of the Klebs-Loeffler-bacillus. *Med. Record.* (Ref. *Baumgarten's Jahresber.*, xvii. 172.)

Russ, Y. (1905), Zur Frage der Bakterizidie durch Alkohol. *Centralbl. f. Bakt.*, xxxvii. 281.

Russell, H. L. (1899), The diphtheria bacillus. *Journ. of Amer. Med. Assoc.*, xxxii. 1427. (Ref. *Baumgarten's Jahresber.*, xv. 257.)

Sacharoff, G. (1905), Ueber Injektionen von Diphtherieantitoxin bei Thieren, welche mit normalem Pferdeserum vorbehandelt waren. *Centralbl. f. Bakt.*, 1 Abt., xxxix. 99.

Sachs, H. (1904), Ueber die Bedeutung des Danysz-Dungernschen Kriteriums, nebst Bemerkungen über Protoxoide. *Centralbl. f. Bakt.*, xxxvii. 251.——(1904), Ueber die Konstitution des Tetanolysins. *Berlin. klin. Wochenschr.*, xl. 412.

Sachse, J. D. W. (1810), *Das wissenswürdigste über die häutige Bräune.* Lübeck.

Sakharoff, N. (1892), Simplification du diagnostic bactériologique de la diphtérie. *Ann. de l'Inst. Past.*, vi. 451.

Salmon, T. W. (21. XI. 1903), The relation of mild types of Diphtheria to the public health. *Med. News*, lxxxiii. 971.——(II. 1904), The present status of the pseudodiphtheria bacillus. *Amer. Journ. Med. Sci.*, cxxvii. 107.——(5. III. 1904), Diphtheria of the vagina, with secondary involvement of the mouth, rectum, and skin. *Amer. Med.*, vii. 391.

Salomonsen, C. J. and Madsen, Th. (1897), Influence de quelques poisons sur le pouvoir antitoxique du sang. *Ann. de l'Inst. Pasteur*, xi. 315.——(XI. 1898), Sur la reproduction de la substance antitoxique après de fortes saignées. *Ibid.* xii. 703.——(III. 1899), Recherches sur la marche de l'immunisation active contre la diphtérie. *Ibid.* xiii. 262.

Salge, B. (1904), Ueber den Durchtritt von Antitoxin durch die Darmwand des menschlichen Säuglings. *Jahrb. f. Kinderheilk.*, x. 1. (Ref. *Centralbl. f. Bakt.*, xxxv. 660.)——(1904), Ueber Immunisirung durch Milch. *Hyg. Rundschau*, xiv. 245.

Salkowski (1898), Ueber die Wirkung der Antiseptica auf Toxine. *Berlin. klin. Wochenschr.*, xxxv. 545.

Salter, A. (1899), The pathogenicity of the pseudo-diphtheria bacillus and its relation to the Klebs-Loeffler organism. *Trans. Jenner Inst. Prev. Med.*, 2nd Ser., p. 113.

Salus, G. (1899), Die bacteriologische Diphtheriediagnose in der Hauspraxis. *Prag. med. Wochenschr.*, p. 456.——(1902), Die bacteriologische Diagnose der Diphtherie. *Ibid.* xxvii. (Ref. *Med. Chronicle*, v. 1902.)

Sanarelli, G. (1892), Der menschliche Speichel und die pathogenen Mikroorganismen der Mundhöhle. *Centralbl. f. Bakt.*, x. 817.

Sanfelice, F. and Malato, V. E. (1903), Studi sul vaiuolo. *Ann. d' Ig. Sper.*, xiii. 1.

Satterthwaite, T. E. and Curtis, E. (1876), *Report of the investigations into the pathogeny of diphtheria.* New York.

Saul, E. (7. III. 1905), Beiträge zur Morphologie der pathogenen Mikroorganismen. Diphtherie- u. Pseudodiphtheriebacillus. *München. med. Wochenschr.*, lii. 446.

Savigne (1891), *Des Altérations du Myocarde dans la Diphtérie.* Lyon.

Scagliosi, G. (1896), Ueber die Veränderungen des Herzmuskels bei Diphtherie. *Virch. Arch.*, cxlvi. 115.

Schabad, J. A. (1900), Die klinische Bakteriologie der Diphtherie. *Jahrb. f. Kinderheilk.*, liv. 381. (Ref. *Hyg. Rundsch.*, xii. 810.)——(1902), Die Diphtherie und der Diphtheriebacillus bei Scharlach. *Arch. f. Kinderheilk.*, xxxiv. (Ref. *Baumgarten's Jahresber.*, xviii. 233.)

Schäfer, E. A. (12. I. 1895), On the persistence of the bacillus of Loeffler after recovery from diphtheria. *Brit. Med. Journ.*, i. 61.

Schaffner (**1845**), *Flora*, p. 501.

Schamschin, W. (**1895**), Beiträge zur Pathologie des Herzmuskels. *Ziegler's Beiträge*, xviii. 47.

Schanz, F. (**6. XII. 1894**), Zur Aetiologie der Diphtherie. *Deutsche med. Wochenschr.*, xx. 920.——(**1896**), Zur Aetiologie der Conjunctivitis pseudomembranosa. *Arch. f. Augenheilk.*, xxxiii. 224. (Ref. *Centralbl. f. Bakt.*, xxi. 224.)——(**1896**), Die Bedeutung des sog. Xerosebacillus bei der Diagnose der Diphtherie. *Berlin. klin. Wochenschr.*, 250.——(**1897**), Die Schnelldiagnose des Loeffler'schen Diphtheriebacillus. *Ibid.* 48.——(**1897**), Zur Differentialdiagnose des Diphtheriebacillus. *Ibid.* 1092.——(**18. VIII. 1898**), Ueber die Pathogenität der Loeffler'schen Diphtheriebacillen. *Deutsche med. Wochenschr.*, xxiv. 522.——(**1898**), Wiederum: das Verhältniss der sogen. Xerosebacillen der Conjunctiva zu den Hofmann-Loeffler'schen Pseudodiphtheriebacillen des Rachens. *Berlin. klin. Wochenschr.*, 674.——(**1898**), Die falschen und echten Diphtheriebacillen. *Wiener med. Presse.* (Ref. *Baumgarten's Jahresber.*, xiv. 253.)——(**1898**), Ueber die Menschen- und Thierpathogenität der Loeffler'schen Diphtheriebacillen. *Ibid.* 2053. (Ref. *Ibid.* xiv. 253.)——(**1899**), Der sogen. Xerosebacillus und die ungiftigen Loeffler'schen Bacillen. *Zeitschr. f. Hyg.*, xxxii. 435.——(**14. I. 1902**), Zu Behring's neuester Diphtherietheorie. *München. med. Wochenschr.*, xlix. 64.

Schauffter, W. G. (**1902**), A new stain for diphtheria bacilli. *Med. Record.* (Rev. *Philad. Med. Journ.*, 1902, p. 909.)

Scheller, R. (**24. XI. 1905**), Beiträge zur Diagnose und Epidemiologie der Diphtheritis. *Centralbl. f. Bakt.*, xl. 1.

Schemm, G. C. (**1890**), Ueber die Veränderungen der Herzmusculatur bei Rachendiphtherie. *Virch. Arch.*, cxxi. 235.

Scheppegrell, W. (**III. 1904**), Diphtheria of the nose. *Amer. Journ. Med. Sci.*, xlii. 437.

Schick, B. and Ersettig, H. (**27. VIII. 1903**), Zur Frage der Variabilität der Diphtheriebacillen. *Wien. klin. Wochenschr.*, xvi. 993.

Schilling (**1902**), Ueber den Pseudodiphtheriebacillus bei akuter Mittelohrenzündung. *Monatsschr. f. Ohrenheilk.*, No. 10. (Ref. *Centralbl. f. Bakt.*, xxiv. 379.)

Schlesinger, E. (**1900**), Die Leucocytose bei experimenteller Diphtherie. *Arch. f. Kinderheilk.*, xxx. 233. (Ref. *Baumgarten's Jahresber.*, xvi. 181.)——(**15. I. 1901**), Ein Beitrag zur Diphtherie der Conjunctiva. *München. med. Wochenschr.*, xlviii. 101.

Schloffer, H. (**18. XI. 1893**), Ueber die Verwendung des Harnagar zur Züchtung des Diphtheriebacillus. *Centralbl. f. Bakt.*, xiv. 657.

Schlossmann, A. (**1894**), Diphtherie und Diphtheriebacillus. *Klin. Zeit- und Streitfragen*, viii. 5. (Ref. *Baumgarten's Jahresber.*, x. 239.)

Schmid and Pflanz (**1896**), Sul potere immunizzante del latte di donna contro la tossina difterica. *Riforma med.*, xii. 253. *Wien. med. Wochenschr.*, xvi.

Schmiegelow (**1903**), Die fibrinöse Rhinitis. *Monatsschr. f. Ohrenheilk.*, xxv. 144. (Ref. *Centralbl. f. Bakt.*, xxxiv. 517.)

Schoedel, J. (**1900**), Mitteilungen aus der städtischen Diphtherieuntersuchungsstation in Chemnitz. *München. med. Wochenschr.* (Ref. *Centralbl. f. Bakt.*, xxix. 512.)——(**1900**), I. Bacilläre Magendiphtherie. Diphtheriebacillen in Magen und Darminhalt und in den Dejektionen. II. Der Joossche Serumagar als Nährboden für Diphtheriebacillen. *Ibid.* xxxvii. 895.

Schottelius, M. (**19. XII. 1896**), Ueber das Wachstum der Diphtheriebacillen in Milch. *Centralbl. f. Bakt.*, xx. 897.

Schottmüller, H. (1895), Ein Fall von Wunddiphtherie mit Diphtheriebacillen bei gleichzeitigen Vorhandensein von Diphtheriebacillen in gesunden Rachen. *Deutsche med. Wochenschr.*, XXI. 272.

Schreiber (1888), Ueber die Bedeutung der sogenannten Xerosebacillen. *Fortschr. d. Med.*, p. 650. (Ref. *Baumgarten's Jahresber.*, IV. 237.)

Schreider, M. v. (26. **VIII**. 1892), Ueber Mischkulturen von Streptokokken und der Diphtheriebacillen. *Centralbl. f. Bakt.*, XII. 289.

Schrevens (1894), Sur les rapports de la diphtérie aviaire avec la diphtérie humaine. *Bull. Acad. roy. de Belg.*, VIII. 380.

Schulze (1898), *Die Serum Exantheme bei Diphtherie.* (*Inaug. Dissert.*, Berlin.)

Schumburg (3. **VI**. 1902), Die Beziehungen der Babes-Ernst'schen Körperchen zu der Virulenz der Bakterien. *Centralbl. f. Bakt.*, Orig. XXXI. 694.

Schütz (4. **IV**. 1898), Zur Frage der Mischinfection bei Lungentuberculose (Diphtherie und diphtherieähnliche Bacillen) in tuberculösen Lungen. *Berlin. klin. Wochenschr.*, XXXV. pp. 297, 335, 356.

Schwab, Th. (1904), Zwei Fälle von ausgedehnten Ulcerationsprozessen an Mund und Genitalien, hervorgerufen durch Diphtheriebacillen. *Arch. f. Dermat. u. Syph.*, LXVIII. (Ref. *Centralbl. f. Bakt.*, XXXV. (Ref.) p. 480.)

Schweighofer (1892), Ueber Sekundärinfection bei Diphtherie. *Oesterr. ärztl. Vereinszeit'g.*, No. 13.

Schwenk, P. N. K. (14. **II**. 1903), Post-diphtheritic ocular paralysis. *Med. News*, LXXXII. 295.

Schwoner, J. (27. **XI**. 1902), Ueber Differenzierung der Diphtheriebacillen von den Pseudodiphtheriebacillen durch Agglutination. *Wien. klin. Wochenschr.*, XV. 1274.——(10. **XII**. 1903), Ein Beitrag zur Kenntniss der Pseudodiphtheriebacillen. *Ibid.* XVI. 1385.——(1904), Ueber die hämolytische Wirkung des Loefflerschen Bacillus. *Centralbl. f. Bakt.*, XXXV. 608.

Seiler, F. and Stoutz, de W. (20. **III**. 1904), *Rev. Méd. de la Suisse Romande.* (Ref. *Med. News*, LXXXIV.)

Seitz, J. (1899), Diphtheriebacillen in einen Panaritium. *Korrespondenzbl. f. Schweiz. Aerzte*, No. 21. (Ref. *Centralbl. f. Bakt.*, XXVII. 279.)

Sellner, B. (14. **X**. 1897), Ueber Diphtheriebacillen beim Scharlach. *Wien. klin. Wochenschr.*, X. 900.

Senator, H. (1872), Ueber Diphtherie. *Virch. Arch.*, LVI. 56.——(1874), Ueber Synanche contagiosa. *Samml. klin. Vorträge*, No. 78.

Seng, W. (1899), Ueber die qualitativen und quantitativen Verhältnisse der Eiweisskörper im Diphtherieheilserum. *Zeitschr. f. Hygiene*, XXXI. 513.

Sennertus, D. (1628), *Practicae medicinae* (liber secundus), p. 94, Witteberg.

Seuvre (1898), Quoted by Cary and Lyon, 1901.

Sevestre (1895), De la persistance du bacille chez les enfants guéris de la diphtérie et des indications qui en résultent au point de vue de l'hygiène publique. *Rev. d'Hyg.*, XVII. 294.

Sevestre and Méry (**III**. 1895), *Rev. Mens. d. Malad. de l'Enfance.* (Review in *Amer. Journ. Med. Sci.*, CX. 116.)

Sharp, G. (22. **VIII**. 1896), Soil in relation to diphtheria and its organism. *Brit. Med. Journ.*, II. 442.——(1897), The action of the products of the organism of diphtheria on the heart muscle of the frog (Rana temporaria). *Journ. of Anat. and Phys.*, XXXI. 199.——(4. **VI**. 1898), Diphtheria affecting the skin. *Brit. Med. Journ.*, I. 1451.——(7. **VII**. 1900), Contagious catarrh or roup in fowls and diphtheria in man. *Lancet*, II. 18.

Shattock, S. G. (1894-5), Experimental observations on some cases of diphtheria, with a note on the division of the bacillus. *Trans. Path. Soc.*, XLVI. 258.——(1898), Experiments to determine whether sewer air will raise the toxicity of lowly virulent diphtheria bacilli. *Journ. of Pathol. and Bacteriol.*, V. 305.

Sholley, A. von L. (15. VI. 1907), A contribution to the statistics on the presence of diphtheria bacilli in apparently normal throats. *Journ. of Infect. Dis.*, IV. 337.

Shuttleworth, E. B. (14. X. 1895), Laboratory notes on the bacteriology of diphtheria. *Lancet*, II. 665.——(1896), Bacteriological diagnosis as governing the admission and discharge of patients in diphtheria hospitals. *Amer. Pub. Health Assoc.*, XXII. 68.

Sieber, N. (1901), Ueber die Entgiftung der Toxine durch die Superoxyde, sowie thierische und pflanzliche Oxydasen. *Zeitschr. f. physiol. Chem.*, XXXII. 573.

Siegert (1900), Vier Jahre vor und nach der Einführung der Serumbehandlung bei Diphtherie. *Jahrb. f. Kinderheilk.*, LII.——(1902), Bemerkung zu Prof. Kassowitz, "Die Erfolge des Diphtherie Heilserums." *Therap. Monatsh.*, XVI.

Silberschmidt, W. (1895), Bakteriologisches über Diphtherie. *München. med. Wochenschr.*, XLII. 185. (Ref. *Hyg. Rundsch.*, V. 848.)

Silberstein, L. (30. VIII. 1900), Ein Fall von Vulvovaginitis diphtheritica. Behandlung mit Heilserum. Heilung. *Deutsche med. Wochenschr.*, XXVI. 566.

Simon, J. (1889), *Nouvelles études sur la diphtérie.* Paris. (Ref. *Baumgarten's Jahresber.*, V. 219.)

Simon, L. G. (1904), Contribution à l'étude anatomo-pathologique de la diphtérie humaine. Lésions et réactions du système hématopoiétique. *Centralbl. f. Bakt.*, Ref. XXXV. 479.

Simoni, A. de (31. VIII. 1898), Ueber einen sporogenen Pseudo-Diphtheriebacillus. *Centralbl. f. Bakt.*, XXIV. 294.——(V. 1899), Ueber das häufige Vorkommen von Pseudodiphtheriebacillen auf der Nasenschleimhaut. *L' Ufficiale Sanitario.* (Ref. *Centralbl. f. Bakt.*, XXVI. 458.)——(20. XII. 1899), Beitrag zur Morphologie und Biologie der Pseudodiphtheriebacillen. *Centralbl. f. Bakt.*, XXVI. 673.——(23. XII. 1899), Beitrag zur Morphologie und Biologie der Pseudodiphtheriebacillen. *Ibid.* XXVI. 757.

Simonin, J. and Benoist, F. (I. 1898), De la diptérie larvée au cours des épidémies ; son diagnostic, sa fréquence et son rôle. *Rev. de méd.*, XVIII. 48.——(5. I. 1898), Sur l'identité des bacilles et des pseudo-bacillus de la diphtérie. *Semaine Méd.*, XVIII. 13. (Meeting Soc. de Biologie, 8. I. 1898.)

Sittler, P. (1906), Uebertragung von Diphtherie durch dritte Personen. *München. med. Wochenschr.*, LIII. 863.

Slawyk and Manicatide (1898), Untersuchungen über 30 verschiedene Diphtheriestämme mit Rücksicht auf die Variabilität derselben. *Zeitschr. f. Hyg.*, XXIX. 181.

Smirnow, G. A. (1888), Ueber Gastritis membranacea und diphtheritica. *Virch. Arch.*, CXIII. 333.——(1894), Ueber die Behandlung der Diphtherie mit Antitoxinen die ohne Vermittlung des thierischen Organismus darstellbar sind. *Berlin. klin. Wochenschr.*, XXXI.——(1895), Ueber die Behandlung der Diphtherie mit künstlich dargestellten Antitoxinen. *Ibid.* XXXII. 645, 675.

Smith, F. (19. XI. 1898), Diphtheria bacilli in urine. *Lancet*, II. 1325.——(I. 1904), What evidence can be adduced as to the conveyance of diphtheria infection from the lower animals? *Journ. Roy. Army Med. Corps*, II. 43.

Smith, H. E. (14. VII. 1900), Conjunctival diphtheria. *Brit. Med. Journ.*, II. 91.

Smith, J. H. (1907), On the absorption of antibodies from the subcutaneous tissues and peritoneal cavity. *Journ. of Hygiene*, VII. 205.

Smith, J. L. (2. VI. 1894), A note on a new method of preparing culture media. *Brit. Med. Journ.*, I. 1177.

Smith, P. C. (16. X. 1897), The etiology of diphtheria with special reference to two localized outbreaks at Wandsworth. *Lancet*, II. 974.

Smith, Th. (1896), The conditions which influence the appearance of toxin in cultures of the diphtheria bacillus. *Trans. Assoc. of Amer. Phys.*, 24 pp. repr. ——(1897), A modification of the method for determining the production of indol by bacteria. *Journ. of Exper. Med.*, II. 542.——(1899), The relation of dextrose to the production of toxin in bouillon cultures of the diphtheria bacillus. *Ibid.* IV. 373.——(II. 1905), Degrees of susceptibility to diphtheria toxin among guinea-pigs. Transmission from parents to offspring. *Journ. Med. Research*, XIII. 341.

Smith, Th. and Walker, E. (1896), A comparative study of the toxin production of diphtheria bacilli. *28th Ann. Rep. State Board of Health of Mass.*, p. 649.

Smith, W. R. (III. 1900), *Journ. of State Med.*, III. 182. (Quoted by Pugh, 1902.)

Smith, W. R. and Somerville, D. (I. 1904), Note on the bacteriological standardization of disinfectants. *Journ. of State Med.*, XII. 44.

Solis-Cohen, M. (6. VI. 1907), Latent diphtheria. *Journ. of Amer. Med. Assoc.*, 30.

Sörensen, S. T. (1886), Om Krup og Tracheotomi. *Nordiskt med. Arkiv*, XVIII. No. 25.——(1892), Ueber Scharlachdiphtheritis. *Zeitschr. f. klin. Med.*, XIX. (Ref. *Baumgarten's Jahresber.*, VIII. 200.)——(1899), Ueber Diphtheriebacillen und Diphtherie in Scharlachabtheilungen. *Zeitschr. f. Hyg.*, XXXI. 265.

Spirig, W. (1899), Ueber die Diphtheriebacillen einer Hausepidemie. *Zeitschr. f. Hyg.*, XXX. 511.——(16. XI. 1899), Die Streptothrix (Actinomyces-) Natur des Diphtheriebacillus. *Centralbl. f. Bakt.*, XXVI. 540.——(1903), Studien über den *Diphtheriebacillus*. *Zeitschr. f. Hyg.*, XLII. 420.

Spronck, C. H. H. (6. IX. 1891), Die Invasion des Klebs-Loeffler'schen Diphtheriebacillus in die Unterhaut des Menschen. (*Centralbl. f. allg. Pathol. u. pathol. Anat.*, III. 339.)——(1895), Sur les conditions dont dépend la production du poison dans les cultures diphtériques. Moyen simple de préparer une toxine très active. *Ann. de l'Instit. Pasteur*, IX. 758–765.——(3. IX. 1896), Ueber die vermeintlichen "schwachvirulenten Diphtheriebacillen" des Conjunctivalsackes und die Differenzirung derselben von dem echten Diphtheriebacillus mittels des Behring'schen Heilserums. *Deutsche med. Wochenschr.*, XXII. 571. ——(1896), Le diagnostic bactériologique de la diphtérie controlé par le sérum antidiphtérique. *Semaine Méd.*, XVI. 317.——(1897), Le diagnostic bactériologique de la diphtérie et les difficultés causés par les bacilles pseudo-diphtériques. *Ibid.* XVII. 353.——(1898), La culture du bacille de Hansen et le sérodiagnostique de la Lèpre. *Ibid.* V. 369.——(X. 1898), Préparation de la toxine diphtérique. *Ann. de l'Inst. Pasteur*, XII. 701.——(X. 1898), Influence favorable du chauffage du sérum antidiphtérique sur les accidents post-sérothérapiques. *Ibid.* XII. 696.

Spronck, C. H. H., Wintgens, E. and van der Brink, J. A. (1889), Die diphtheriebacil (Klebs-Loeffler) en zijn pathogene beteekenis. *Nederl. Tijdschr. v. Geneesk.*, XXV. p. 685.

Stahl, B. F. (1898), A case of diphtheria of the uterus. *Trans. Path. Soc. Philadel.*, XVIII. 296. (Quoted by Salmon, 5. III. 1904.)

Stamm, C. (1892), Die Aetiologie der Rhinitis pseudomembranacea. *Arch. f. Kinderheilk.*, XIII. (Ref. *Centralbl. f. Bakt.*, XII. 673.)

Starr (1752), An account of morbus strangulatorius. *Phil. Trans.*, XLVI. 435.

Stecksén, A. (23. III. 1900), Experimentelle Studien über die ätiologische Bedeutung des Loeffler'schen Diphtheriebacillus. *Centralbl. f. Bakt.*, XXVII. 389.

Steensland, H. S. (1901), Acute degeneration of the nervous system, the muscles, and the heart. *Med. a. Surg. Reports Boston City Hospital*, XII. 159.

Steffens, P. (1900), Ein Fall von Lidgangrän mit Diphtheriebacillenbefund. *Klin. Monatsbl. f. Augenheilk.*, p. 339. (Ref. *Baumgarten's Jahresber.*, xvii. 193.)

Stein, W. (15. XII. 1900), Zur Bakteriologie der Ozoena. *Centralbl. f. Bakt.*, xxviii. 726.

Stejskal, K. v. (1902), Kritisch experimentelle Untersuchungen über Herztod in Folge von Diphtherietoxin. *Zeitschr. f. klin. Med.*, xliv. 222.

Stephens, J. W. W. and Parfitt, C. D. (1897), Three cases of haemorrhagic diphtheria. *Journ. of Pathol. and Bacteriol.*, iv. 424.

Stephens, J. W. W. and Wood Smith, R. F. (30. VI. 1896), Vibrio tonsillaris (Klein). Beschreibung eines aus der Mundhöhle isolierten Vibrio. *Centralbl. f. Bakt.*, xix. 929.

Stephenson, S. (1898), On epithelial xerosis of the conjunctiva. *Trans. Ophthal. Soc. of United Kingdom*, xviii. 55.——(15. VI. 1898), The diagnosis of diphtheria of the conjunctiva. *Brit. Med. Journ.*, i. 1578.——(28. I. 1899), Three cases of diphtheritic conjunctivitis. *Lancet*, i. 227.——(17. II. 1900), Diphtheria of the conjunctiva. *Ibid.* i. 451.

Sternberg, G. M. (1889), Bacillus diphtheriae (Loeffler). *Brooklyn Med. Journ.*, iii. 145.——(1901), *Text-Book of Bacteriology*, 2nd ed., p. 452.

Stevenson (VII. 1898), *Journ. of Comp. Med.* (Quoted by Harrison, xii. 1903.)

Stoddardt, F. W. (1895), The bacterioscopic diagnosis of diphtheria. *Bristol Med. Chir. Journ.*, xiii. 18.

Stöhr, P. (1884), Ueber Mandeln und Balgdrüsen. *Virchow's Arch.*, xcvii. 211.

Stokes, W. R. (12. XII. 1895), The bacteriological examination of nine autopsies on cases of diphtheria treated with antitoxin. *Boston Med. and Surg. Journ.*, cxxxiii. 581.——(VII. 1901), Ulcer of the stomach caused by the diphtheria bacillus. *Johns Hopkins Hosp. Bull.*, xii. 208.

Stone, A. K. (XI. 1897), Virulence of bacilli in cases of diphtheria after the disappearance of clinical symptoms. *Journ. of Boston Soc. of Med. Sci.*, ii. 11.

Stone, H. B. (13. VIII. 1904), A new pathogenic throat organism. *Med. Record*, lxvi. (Ref. *Amer. Med.*, viii. 340.)

Stooss (1901), Das regelmässige Vorkommen von Diphtherie- oder Pseudodiphtheriebacillen in den gewöhnlichen Schnupfen der Kinder. *Arch. f. Kinderheilk.*, xxxii. 401.

Strack, H. L. (1894), Einleitung in den Talmud. 2. Auflage, Leipzig (und briefliche Mitteilung).

Strasburger, J. (1897), Ueber die Virulenz der Diphtherie in Bonn. *Zeitschr. f. Hyg.*, xxv. 389.

Straus (1888), Sur l'absence de microbes dans l'air expiré. *Ann. Inst. Pasteur*, ii. 181.

Streit, H. (1904), Untersuchungen über die Geflügeldiphtherie. *Zeitschr. f. Hyg.*, xlvi. 407.

Strelitz, E. (1891), Zur Kenntniss der im Verlaufe von Diphtherie auftretenden Pneumonien. *Arch. f. Kinderheilk.*, xiii. 468, 481.

Stromeyer (1873), *Centralbl. f. d. med. Wiss*, xi. No. 21.

Strsheminsky, J. J. (1897), Ein Fall von pseudomembranösen Augenbindehautentzündung hervorgerufen durch den Loeffler'schen Bacillus und geheilt mit Behring's Heilserum. *Wratsch.*, p. 161. (Ref. *Centralbl. f. Bakt.*, xxi. 552.)

Stubbs, W. P. (X. 1904), Disinfection in diphtheria and scarlet fever in Baltimore with an account of reinfections. *Maryland Med. Journ.* (Ref. *Amer. Med.*, viii. 1023.)

Sudeck, P. (1896), Ueber das Vorkommen von diphtherie-ähnlichen Bacillen in der Luft. *Festschr. z. Feier des 80-jähr. Stiftungsfestes d. ärztl. Ver. zu Hamburg* (A. Langkammer). (Ref. *Centralbl. f. Bakt.*, xxiii. 552.)

Sudsuki, K. (1901), Ueber die Pathogenese der diphtherischen Membranen. *Ziegler's Beitr. z. pathol. Anat.*, xxix. 562.

Süsswein, J. (6. II. 1902), Das Schicksal der Diphtheriebacillen im Verdauungscanale und die dasselbe bestimmenden Factoren. *Wien. klin. Wochenschr.*, xv. 148.

Sutton, H. G. (13. I. 1894), Diphtheria and its causation. *Lancet*, i. 89.

Swieten, G. van (1745), *Commentaria in Hermanni Boerhaave aphorismos de cognoscendis et curandis morbis.* Lugduni Batavorum, ii. 9 S. 683.

Swithinbank, H. and Newmann, G. (1903), *Bacteriology of Milk* (John Murray), p. 336.

Symes, J. O. (24. VIII. 1895), Note on the bacteriological examination of the throats in some fevers. *Lancet*, ii. 455.——(6. VI. 1896), Diphtheria in cats. *Brit. Med. Journ.*, i. 1385.——(23. II. 1903), The presence of diphtheria bacilli in atrophic rhinitis. *Ibid.* ii. 484.

Szontagh, F. v. and Wellmann, O. (1898), Vergleichende chemische Untersuchungen über das normale Pferdeserum und das Diphtherieheilserum. *Deutsche med. Wochenschr.*, xxiv. 421.

Talamon, C. (1881), Note sur le microbe de la diphtérie. *Bull. Soc. anat. de Paris*, lvi. 44 ; *Progrès méd.*, pp. 122, 496.

Tangl, F. (1890), Untersuchungen über Diphtherie. *Centralbl. f. allg. Path. u. path. Anat.*, i. 795.——(1891), Studien über die menschliche Diphtherie. *Arb. a. d. Gebiete der pathol. Anat. herausg. von Baumgarten*, i. 85.——(8. VII. 1891), Zur Frage der Scharlachdiphtheritis. *Centralbl. f. Bakt.*, x. i.

Tappeiner, H. and Jodlbauer, A. (1904), Ueber die Wirkung fluoreszierender Stoffe auf Diphtherietoxin und Tetanustoxin. *München. med. Wochenschr.*, li. 737.

Tavel (1901), Ueber Wunddiphtherie. *Deutsche Zeitschr. f. Chir.*, lx. 460. (Ref. *Baumgarten's Jahresber.*, xvii. 193.)

Teich, M. (1899), Beiträge zur Kultur des Leprabacillus. *Centralbl. f. Bakt.*, xxv. 756.

Teisser, J. and Guinard, L. (1897), Recherches expérimentales sur les effets des toxines microbiennes et sur quelques influences, etc. *Arch. de méd. expér.*, ix. 994.

Tertsch (1903), Ein Beitrag zu den dem Diphtheriebacillus ähnlichen Stäbchen des Konjunktivalsackes. *Zeitschr. f. Augenheilk.*, xiii. 621.

Testi (1902), Azione dei geli e disgeli alternati sulla vitalità e virulenza di alcuni batteri patogeni. *Riforma Med.*, No. 41. (Ref. *Centralbl. f. Bakt.*, xxxiii. 17.)

Tézenas du Montcel (5. VIII. 1893), Durée de la période contagieuse chez les diphtériques convalescents d'après l'examen bactériologique. *Province Méd.*, vii. 361. (Ref. *Hygien. Rundsch.*, iv. 133.)

Thaon, L. (1885), Des broncho-pneumonies infectieuses de l'enfance et de leurs microbes. *Compt. rend. Soc. Biol.*, Paris, ii. 617–620.

Thiercelin, Quoted by *Jurist* in *Journ. of Amer. Med. Assoc.*, 1898 ; requoted by Trevelyan, 14. IV. 1900.

Thomas, C. J. (VII. 1904), School diphtheria in the Metropolis. *Brit. Med. Journ.*, ii. 431.

Thomas, J. J. (27. I. 1898), Acute degenerations of the nervous system in diphtheria. *Boston Med. and Surg. Journ.*, cxxxviii., pp. 76, 97 and 123 ; also *Med. a. Surg. Rep. Boston City Hosp.*, ix. 52.

Thomas, J. J. and Hibbard, C. M. (1900), Heart-failure in diphtheria. *Med. a. Surg. Rep. Boston City Hosp.*, xi.

Thomson, St C. and Hewlett, R. T. (1895), Micro-organisms in the healthy nose. *Med.-chirurg. Trans.*, LXXVII. 239.

Thorne, R. T. (1891), Abstracts of the Milroy lectures on diphtheria; its natural history and prevention. *Brit. Med. Journ.*, pp. 395–396; also "Diphtheria." Milroy lectures.

Thursfield, W. N. (VIII. 1878), On diphtheria from a preventive medicine point of view. *Lancet*, II. 145, 179, 210, 311.

Tigri (I. 1867), *Bull. Acad. de méd.*

Tizzoni, G. and Centanni, E. (1893), La trasmissione ereditaria da padre a figlio dell' immunità contro la rabbia. *Riforma med.*

Tobey, E. N. (XII. 1906), Non-virulent diphtheria bacilli from infected middle ear. *Journ. med. Research*, XV. 319.

Tobiesen, F. (31. X. 1892), Ueber das Vorhandensein des Loeffler'schen Bacillus im Schlunde bei Individuen, welche eine diphtherische Angina durchgemacht haben. *Centralbl. f. Bakt.*, XII. 587.

Tochtermann, A. (1895), Ein aus Blutserum gewonnener sterilisirbarer Nährboden, zugleich ein Beitrag zur Frühdiagnose der Diphtherie. *Centralbl. f. innere Med.*, XVI. 961. (Ref. *Hyg. Rundsch.*, VI. 359.)

Todd, C. (28. V. 1898), A form of external rhinitis due to the Klebs-Loeffler bacillus appearing in children convalescent from scarlet fever. *Lancet*, I. 1458.

Tourenhout, H. F. van (1895), *Over bereiding van diphtheriegif.* Dissertation, Utrecht. (Ref. *Centralbl. f. Bakt.*, XVIII. 295.)

Townsend, C. W. (1894), Primary nasal diphtheria. *Boston Med. and Surg. Journ.*, CXXX. 513.

Trambusti, A. (1896), Ricerche etiologiche sul midollo delle osse nella difterite. *Pubbl. d. R. Istit. di Studi superiori Firenze.* (Ref. *Centralbl. f. Bakt.* XXV.)

Treitel and Koppel (1895), Ueber rhinitis fibrinosa. *Arch. f. Kinderheilk.*, XVIII. 107. (Ref. *Baumgarten's Jahesber.*, XI. 266.)

Trendelenburg (1869), Ueber die Contagiosität und die locale Natur der Diphtheritis. *Arch. f. klin. Chir.*, X. 720.

Trevelyan, E. F. (25. I. 1896), Some points in the pathology of diphtheria *Lancet*, I. 226.—— (14. IV. 1900), On Diphtherial Stomatitis. *Brit. Med. Journ.*, I. 898. ——(19. V. 1900), *Lancet*, I. 1443. [Report of meeting of Manchester Path. Soc.]

Troje, G. (1892), Ueber spontane und experimentelle Perlsucht. *Berl. klin. Wochenschr.*, No. 9.

Trousseau (1828), *Cliniques médicales. Rapport sur l'épidémie de Bologne.*—— (1828), *Clinique médicale de l'Hôtel Dieu de Paris*, I.

Trummp, J. (15. XI. 1896), Diphtherie- oder Pseudodiphtherie-Bacillen im Empyemeiter. *Centralbl. f. Bakt.*, XX. 721.

Tschiglaw (1887), Ueber die pathologische Veränderungen der Nieren bei Diphtherie. *St Petersb. med. Wochenschr.*

Tulp, N. (1685), *Observationes medicae.* [Amsterdam.] Lib. IV. Cap. 9, 294, Membrana laryngis expectorata.

Tunnicliffe, R. (2. III. 1906), The identity of fusiform bacilli and spirilla. *Journ. Infect. Dis.*, III. 148.

Turner (1887), *Brit. Med. Journ.*, II. 416 (Editorial).

Turner (1904), Diphtheria statistics. *New Engl. Med. Gaz.*, Boston, XXXIX.

Turner, G. (7. IV. 1900), Diphtheria in men and gapes in birds. *Brit. Med. Journ.*, I. 879. (Letter.)

Tyler, G. F. (5. I. 1901), The relation of state and local boards of health to outbreaks of diphtheria. *Philad. Med. Journ.*, VII. 42.

Uffenheimer, A. (1904), [Reviews Salge's (1904) paper.] *Centralbl. f. Bakt., Referate* xxxv. 660.

Uhthoff, W. (1902), Diphtherie der menschlichen Conjunctiva. *Sitz.-Ber. d. Ophthal. Ges. Heidelberg*, p. 282. (Ref. *Baumgarten's Jahresber.*, xviii. 234.)

Ulmann, G. and Oppenheim, R. (31. VIII. 1898), Persistance du bacille de Loeffler dans la gorge des sujets atteints de diphtérie. *Presse Méd.* (Ref. *Centralbl. f. innere Med.*, xx. 792.)

Unruh (1883), Ueber Myocarditis bei Diphtherie. *Jahrb. f. Kinderheilk.*, xx. 1.

Uschinsky, N. (1893), Ueber eine eiweissfreie Nährlösung für pathogene Bakterien nebst einige Bemerkungen über Tetanusgift. *Centralbl. f. Bakt.*, xiv. 316.——(6. II. 1897), Ueber Diphtheriekulturen auf eiweissfreier Nährlösung. *Ibid.* xxi. 146.

Uthoft (1896), *Berlin. klin. Wochenschr.*, No. 12. (Quoted by Schanz, 1894.)

Vaillard, L. (1896), Sur l'hérédité de l'immunité acquise. *Ann. Inst. Pasteur*, x. 65.

Valagussa, F. and Ranelletti, A. (24. XI. 1898), Untersuchungen über die Wirkung des Diphtheriegifts in ihrer Beziehung zu den Lebenbedingungen des Organismus. *Ann. d' Ig. Sper.*, ix. (Autorref. in *Centralbl. f. Bakt.*, xxiv. 752.)

Valude (1894), Conjonctivites à fausses membranes et diphtérie oculaire. *La Semaine Méd.*, xiv. 22.

Vedel, A. and V. (1898), Angines à pneumocoques. *Nouv. Montpellier Méd.*, vii. 621. (Quoted by Cary and Lyon 1901.)

Vedora, T. della (1898), La diagnosi differenziale fra il bacillo di Loeffler ed i simili. *Gazz. d. ospedali*, No. 97. (Ref. *Centralbl. f. inn. Med.*, xx. 791); also (1899) *Arch. ital. di otol.*, p. 58.

Veeder, M. A. (14. II. 1901), Disinfection within or without the body in diphtheria. *Boston Med. and Surg. Journ.*, cxliv. 153.

Veillon (1893), La diphtérie; étude bactériologique; applications au diagnostic, à la pathogénie et au traitement. *Semaine Méd.*, xiii. 436.——(1894), Recherches sur l'étiologie et la pathogénie des angines aigues non diphtéritiques. *Arch. de méd. exp. et d'anat. path.*, vi. 161. (Ref. *Hyg. Rundsch.*, iv. 1125.)

Velde, H. van de (1897), Beitrag zur Kenntniss der antitoxischen und antiinfectiösen Kraft des Antidiphtherieserums. *Centralbl. f. Bakt.*, xxii. 527.

Verey, S. Artault de (26. X. 1895), Deux cocs diphtériques traités par le sérum de Roux. *Compt. rend. Soc. de Biol.*, ii. 683.

Veszprémi, D. (1905), Kultur- und Tierversuche mit dem Bacillus fusiformis und dem Spirillum. *Centralbl. f. Bakt.*, xxxviii. 136.

Vicquerat (1902), Toxin und Isomerie. *Centralbl. f. Bakt.*, xxxi. 581.

Vieusseux, G. (1806), Observations sur le croup. *Journ. de méd. de Corvisart, Leroux et Boyer*, xii. 422. (Paris.)——(1812) *Mémoire sur le croup ou angine trachéale* qui a obtenu la première mention honorable au concours ouvert par S. M. l'Empereur sur cette maladie. (Geneva.)

Vincent, H. (IX. 1896), Sur l'étiologie et sur les lésions anatomopathologiques de la pourriture d'hôpital. *Ann. de l'Inst. Past.*, x. 488.——(1898), Sur une forme particulière d'angine diphtéroide (angine à bacilles fusiformes). *Arch. internat. de Laryngol.* (Ref. *Hyg. Rundsch.*, ix. 208.)——(VIII. 1899), Recherches bactériologiques sur l'angine à bacilles fusiformes. *Ann. de l'Inst. Pasteur*, xiii. 609.——(1894), Sur les altérations du plexus cardiaque dans la paralysie du cœur consécutive à la diphtérie. *Arch. de Méd. expér.*, vi. 513.

Vincenzi, L. (1898), Sulla presenza del bacillo difterico nell' acqua santa e dell' acqua santa come mezzo di trasmissione di malattie infettive. *Arch. per le Sci. Med.*, xxii. (Ref. *Baumgarten's Jahresber.*, xiv. 310.)

Virchow, R. (1847), *Virchow's Archiv*, vol. I.——(1856), *Gesammelte Abhandlungen zur wissenschaftlichen Medizin.*——(1854), *Handbuch der spez. Pathologie und Therapie.*

Virey (1901), *Journ. de méd. et de chir. prat.*, LXXII. 74.

Vogelsberger (1904), [Antidiphtheritic serum.] *Inaug. Dissert.*, Berlin.

Vogt (1895), Om den bakteriologiske diagnose ved Diftéri. *Norsk. Mag. f. Laegevidensk.*, R. 4, X. 184.

Vossius, A. (1901), Ein Beitrag zur Lehre von der Aetiologie, Pathologie und Therapie der Diphtheritis conjunctivae. *Deutsche Praxis*, III. (Ref. *Hyg. Rundsch.*, XI. 537.)

Wachenheim, F. L. (1898), The clinical relations of the Loeffler bacillus. *New York Med. Journ.*, LXVII. 858.

Waelsch, L. (1899), Ueber einen Bakterienbefund bei Pemphigus vegetans nebst Bemerkungen zur Differential-diagnose zwischen Diphtherie- und pseudodiphtheriebacillen. *Arch. f. Dermatol. u. Syph.*, L. 71.

Wagner, E. (1866), Die Diphtheritis und der Croup des Rachens und der Luftwege in anatomischer Beziehung. *Arch. d. Heilk.*, VII. 481.——(1880), Beiträge zur Kenntniss der acuten morbus Brightii. *Deutsches Arch. f. klin. Med.*, XXV.; *v. Ziemssens Handbuch*, IX. 1 u. 2. Aufl.

Waldo (1893), *Modern Meteorology.*

Walley, T. (27. VIII. 1887), Diphtheria in man and the lower animals. *Lancet*, II. 437.

Walsh, J. E. (18. VI. 1898), Diphtheria. *New York Med. Journ.*——(VI. 1901), Diphtheria bacilli in Noma. *Proc. Pathol. Soc. Philad.* (Ref. *Baumgarten's Jahresber.*, XVII. 173.)

Walter, K. (1896), Zur Bedeutung des Formalins bezw. Formaldehyds als Desinfectionsmittel. *Zeitschr. f. Hyg.*, XXI. 421.

Ware, E. E. (10. II. 1900), A case of diphtheria of the vulva. *Lancet*, I. 382.

Warnecke, W. (1900), Befund von Xerosebacillen bei progredienter Phlegmone, sekundärer Wundinfektion und Otitis interna. *München. med. Wochenschr.*, p. 1412.

Warry, K. (17. VIII. 1895), Diphtheria and speaking tubes. *Brit. Med. Journ.*, II. 436.

Waschkewitsch, T. (1900), Ueber grosszellige Heerde in den Milzfollikeln bei Diphtheritis und anderen Affectionen. *Virchow's Archiv*, CLIX. 137.

Washbourn, J. W. and Hopwood, E. O. (19. I. 1895), Cases illustrating the importance of an examination for the diphtheria bacillus. *Brit. Med. Journ.*, I. 120.

Wassermann, A. (1894), Ueber Concentrirung des Diphtherieantitoxins aus der Milch immunisierter Tiere. *Zeitschr. f. Hygiene*, XVIII. 235.——(1895), Ueber die persönliche Disposition und die Prophylaxe gegenüber Diphtherie. *Ibid.* XIX. 408.——(1896), Experimentelle Untersuchungen über einige theoretische Punkte der Immunitätslehre. *Ibid.* XXII. 263.——(1898), Ueber ein neue Art von künstlicher Immunität. *Berlin. klin. Wochenschr.*, XXXV. 4.——(1901), Experimentelle Beiträge zur Kenntnis der natürlichen und künstlichen Immunität. *Zeitschr. f. Hyg.*, XXXVII. 173.——(30. X. 1902), Ueber eine neue Art von Diphtherieserum. *Deutsche med. Wochenschr.*, XXVIII. 785.——(1904), A discussion of several new points concerning the theory and practice of immunity. *New York Med. Journ.*, LXXX.

Wassermann, A. and Bruck, C. (1904), Ueber die Wirkungsweise der Antitoxine im lebenden Organismus. *Deutsche med. Wochenschr.*, XXX. 764.

Wassermann and Proskauer (1891), Ueber die von den Diphtheriebacillen erzeugten Toxalbumine. *Deutsche med. Wochenschr.*, XVII. 585.

Wassermann and Takaki (1898), Ueber tetanusantitoxische Eigenschaften des normalen Centralnervensystems. *Berlin. klin. Wochenschr.*, xxxv. 5.

Watson, I. A. (**VII. 1902**), Diphtheria from a bacteriological point of view. *Ann. of Gynecol. a. Pediatr.*

Weaver, G. H. and Tunnicliffe, R. (**24. VI. 1905**), The occurrence of fusiform bacilli and spirilla in connection with morbid processes. *Journ. of Infectious Diseases*, ii. p. 446.

Webster, N. (**1799**), *A brief History of epidemic and pestilential diseases, etc.*, i. p. 143, Hartford.

Weichardt, W. (**1900**), *Die Verbreitung der Diphtherie durch leblose Objekte.* Dissert. Breslau. (Ref. *Hyg. Rundsch.*, xiv. 19.)

Weigert, C. (**1877**), Ueber Croup und Diphtheritis. *Virchow's Arch.*, lxx. 461. ——(**1878**), Ueber Croup und Diphtherie. *Ibid.* lxxii. 218.——(**1879**), Die Bright'sche Nierenerkrankung vom pathologischanatomischen Standpunkte. *Volkmannische Vorträge*, No. 162–3.——(**1880**), Ueber die pathologischen Gerinnungsvorgänge. *Ibid.* lxxix. 87.

Welch, W. H. (**16. V. 1891**), The causation of diphtheria. *Med. News.*——(**X. 1894**), Bacteriological investigations of diphtheria in the United States. A report on behalf of the American Committee on diphtheria to the Eighth International Congress of Hygiene and Demography held in Budapest. *Amer. Journ. Med. Sci.*, N.S. cviii. 35 pp. repr.——(**VII. 1895**), The treatment of diphtheria by antitoxin. *Johns Hopkins Hosp. Bull.*, No. 52, p. 97.

Welch, W. H. and Abbott, A. C. (**1891**), The etiology of diphtheria. *Johns Hopkins Hosp. Bull.*, No. 11, p. 25.

Welch, W. H. and Flexner, S. (**VIII. 1891**), The histological changes in experimental diphtheria. *Johns Hopkins Hosp. Bull.*, ii. 107.——(**III. 1892**), The histological lesions produced by the tox-albumen of diphtheria. *Ibid.* iii. 17.

Wernicke (**1893**), Ein experimenteller Beitrag zur Kenntniss des Loeffler'schen Diphtheriebacillus und zur Blutserumtherapie. *Arch. f. Hyg.*, xviii. p. 192. ——(**1895**), Ueber die Vererbung der künstlich erzeugten Diphtherie-Immunität bei Meerschweinchen. *Festschr. z. 100-jähr. Stiftungsfeier des med.-chirurg. Friedrich Wilhelm-Institutes*, p. 525.

Wesbrook, F. F. (**1903**), Problems in the Laboratory Study of Diphtheria. *Public Health, Rep'ts. and Papers American Pub. Health Assoc.*, xxxvii. 381.——(**25. III. 1905**), Diphtheria infection in Minnesota. Recent experiences with the disease in school children and in institutional epidemics. *Journ. Amer. Med. Assoc.*, xliv. 939.

Wesbrook, F. F., Wilson, L. B. and McDaniel, O. (**1899**), Studies on the distribution of certain varieties of the diphtheria bacillus. *Public Health, Rep. and Pap. Amer. P. Health Assoc.*, xxv. 546.——(**10. I. 1900**), Studies upon the distribution of certain varieties of the diphtheria bacillus. *Journ. Boston Soc. Med. Sci.*, iv. 75.

Wesbrook, F. F., Wilson, L. B., McDaniel, O. and Adair, J. H. (**16. IV. 1898**), A preliminary communication on Bacillus diphtheriae and its variants in a school in which diphtheria was endemic. *Brit. Med. Journ.*, i. 1008.

Wethered (**24. III. 1894**), Diagnosis of diphtheria by bacteriological cultures. *Lancet*, i. 742. (Report London Med. Soc. Meeting.)

Wharton, R. H. (**13. IV. 1895**), A case of glossitis in which the membrane covering the tongue contained Klebs-Loeffler bacilli. *Med. News*, lxvi. 407.

Wheaton, S. W. (**1894**), On certain affections of the mucous membranes and their relation to diphtheria. *Trans. Epidemiol. Soc.*, xiii. 83.

White, C. P. (**1895**), The cultivation of Bacillus diphtheriae on hydrocele fluid. *Trans. Path. Soc. London*, xlvi. 296.

White, F. W. (**29. VIII. 1901**), An apparent case of diphtherial infection from well persons carrying diphtheria bacilli. *Boston Med. and Surg. Journ.*, cxlv. 241.

White, F. W. and Smith, H. H. (**1904**), Heart complications in diphtheria. *Med. Communic. Mass. Med. Soc.*, xix. 923.

Wiener, E. (**1896**), Die Vibrioneninfektion per os bei jungen Katzen und Kaninchen. *Centralbl. f. Bakt.*, xix. 205, 595.

Wier, van (**1660**), *Observationes*, lib. i. § 3 Opp. Amstelod.

Williams, A. (**1898**), *Proc. New York Path. Soc.*, p. 171.——(**V. 1902**), The morphology of the diphtheria bacillus. *Ibid.* i. 4.——(**VI. 1902**), Persistence of varieties of the Bacillus diphtheriae and of Diphtheria-like Bacilli. *Journ. Med. Research*, viii. 83.

Williams, E. H. (**3. XII. 1896**), The prevention of the spread of diphtheria by means of the bacterial test. *Boston Med. and Surg. Journ.*, cxxxv. 582.——(**21. XII. 1901**), Post-scarlatinal diphtheria and rhinorrhœa and otorrhœa. *Brit. Med. Journ.*, ii. 1799.

Williams, F. H. (**XI. 1893**), Diphtheria and other membranous affections of the throat. *Amer. Journ. Med. Sci.*, p. 519.——(**VI. 1895**), Observations on the diagnosis of diphtheria. *Ibid.* cx. 37.

Williams, J. W. (**1898**), *Amer. Journ. of Obstetr.*, xxxviii. 180. (Quoted by Salmon, 5. III. 1904.)

Williams, P. W. (**16. IX. 1905**), What is notifiable diphtheria? *Brit. Med. Journ.*, ii. 647.

Williams, W. (**13. VII. 1895**), Cats and diphtheria. *Brit. Med. Journ.*, ii. 74.——(**VIII. 1898**), Diphtheria of the vulva. *Amer. Journ. of Obstetr.* (Ref. *Med. Chronicle*, x. 47.)

Wilner, A. S. (**3. XI. 1904**), Primary nasal diphtheria; a plea for its early recognition and a report of three cases. *New York Med. Journ.*, lxxx. 881.

Wilson, A. (**1891**), Micro-organism of diphtheria with experimental results in animals. *Trans. Path. Soc. London*, xlii. 346.

Wirgin, G. (**1902**), Zur Wirkung des Aethylalkohols auf Mikroorganismen. *Zeitschr. f. Hyg.*, xl. 307.

Wladimiroff, M. J. (**1895**), Contribution à l'étude du rôle du lait dans l'étiologie de la diphtérie. *Arch. d. Sci. biol. de St Pétersb.*, iii. 85. (Ref. *Baumgarten's Jahresber.*, xi. 209.)

Woakes, E. (**1883**), The etiology of diphtheria, the nature of its contagium, &c. *Lancet*, i. 446.

Wolff (**1899**), *Beitrag zur Lehre von der Rhinitis fibrinosa sive pseudomembranacea.* (*Dissert.* Leipzig.) (Ref. *Centralbl. f. Bakt.*, xxxii. 269.)

Wolff, M. (**1895**), Die Nebenhöhlen der Nase bei Diphtherie, Masern und Scharlach. *Zeitschr. f. Hyg.*, xix. 225.

Wolff, L. (**12. I. 1905**), Ueber die Beziehung der Rhinitis fibrinosa zur Diphtherie. *Deutsche med. Wochenschr.*, xxxi. 65.

Wood, G. E. C. (**1896**), A method for rapidly producing diphtheria antitoxins. *Lancet*, i. 980.

Wood, H. C. and Formad, H. F. (**21. I. 1882**), Report on diphtheria. *National Board of Health Bull. Suppl.*, No. 17.

Woodhead, G. S. (**1895**), Remarks in "A discussion on the pathology of diphtheria and the antitoxic treatment." *Trans. Path. Soc. London*, xlvi. 309.——(**1901**), Report of the bacteriological diagnosis and the antitoxic serum treatment of cases admitted to the hospitals of the Board during the years 1895 and 1896. *Metropol. Asylums Board*, 271 pp., London.

Wright, J. H. (1894), Studies in the pathology of diphtheria. *Bost. Med. a. Surg. Journ.*, CXXXI. 329.——(1895), Studies in the pathology of diphtheria. *Ibid.* CXXXII.

Wright, J. H. and Emerson, H. C. (18. IX. 1894), Ueber das Vorkommen des Bacillus diphtheriae ausserhalb des Körpers. *Centralbl. f. Bakt.*, XVI. 412.

Wright, J. H. and Stokes, W. R. (1895), A report on the bacteriological investigations of autopsies. *Med. a. Surg. Rep'ts. Boston City Hosp.*, VI. 211.——(21. III. 1895), *Boston Med. a. Surg. Journ.* (quoted by Pearce, 1898).

Wünsche, A. (1886), Der Babylonische Talmud in seinen haggadischen Bestandteilen wortgetreu übersetzt und durch Noten erläutert. Leipzig: Otto Schulze.

Wurtz and Bourges (1890), Recherches bactériologiques sur l'angina pseudo-diphtérique de la scarlatine. *Arch. de méd. expér. et d'anat. pathol.*, No. 3. (Ref. *Centralbl. f. Bakt.*, VIII. 301.)

Wurtz, R. and Lermoyez, M. (1893), Du pouvoir bactéricide du mucus humain et en particulier du mucus nasal. *Compt. rend. Soc. de Biol.*, L. 756.

Wyssokowitsch (1886), quoted by Flügge, C. *Die Mikroorganismen*, p. 228.

Zarniko, C. (2. VIII. 1889), Zur Kenntniss des Diphtheriebacillus. *Centralbl. f. Bakt.*, VI. 153, 177, 224.

Zaufal, G. (1895), Ein Beitrag zur Kasuistik der echten Diphtherie der Haut. *Prag. med. Wochenschr.*, No. 10. (Ref. *Centralbl. f. inn. Med.*, XVI. 182.)

Zielleczky, R. (5. XI. 1902), Biochemische und differentialdiagnostische Untersuchungen einiger Bakterien mittels Phenolphtaleinnährböden. *Centralbl. f. Bakt.*, XXXII. 752.

Zimmer, E. (21. IV. 1892), Untersuchungen über das Zustandekommen der Diphtherie-immunität bei Thieren. *Deutsche med. Wochenschr.*, XVIII. 350.

Zucker (1904), Diphtherie mit und ohne Antitoxin behandelt. *Wiener klin. Wochenschr.*, XVII.

Zupnik, L. (1897), Ueber Variabilität des Diphtheriebacillus. *Berlin. klin. Wochenschr.*, XXXIV. 1085.——(1902), Zur Aetiologie der Diphtherie. *Prag. med. Wochenschr.*, No. 30.

Zuppinger (1904), Ueber den Werth der Schutzimpfung gegen Diphtherie. *Wien. klin. Wochenschr.*, XVII.

INDEX.

Abrin 461, 497
Abscesses, secondary to diphtheria 101, 116
  diphtheria bacilli non-virulent in 239
    virulent in 373–375
    virulence not tested 368, 374
  diphtheria-like bacilli in 351, 368–372, 376, 381, 387
  other organisms in 116
Adrenals, diphtheritic lesions of (man) 113
  diphtheritic lesions of (animals) 267, 270
Adsorption theory (Bordet) 564
Aetius 4
Agglutination experiments
  diphtheria bacilli 226, 572
  diphtheria-like bacilli 228
  Hofmann's bacilli 227
Air, diphtheria bacilli in 320
Air-borne diphtheria 320
Alligator, susceptibility to toxin 452
American diphtheria bacillus 462
"Anaphylaxia" 627
Andrade's stain 145
Animal and human diphtheria 31, Chapter VII
Animals, diphtheria bacilli in 285, 288
  diphtheria-like bacilli in 281, 291, 292
  effects of injections of heterologous sera 626, 628
  susceptibility to toxin 471
Anti-antitoxin, production of 568, 638
Anti-microbial diphtheria serum 571
Antiseptics, effects on cultures 170
  effects on bacilli in mouth 422
ANTITOXIN
  absorption from subcutaneous tissue 565
  action in clinical cases 566
  alimentary canal, absorption from 567
  anti-antitoxin, production of 568, 638
  cold, effects on 519
  collapse, due to 636, 637
  concentration of 527
    maximum in blood 515
  curative strength of 525

ANTITOXIN (*cont.*)
  death, sudden, following 636
  disappearance from blood, rate of 566
  discovery of 48, 500
  extent to which used 611
  filtration of 520
  gases, effects on 519
  heat, effect on 518
  horses, in blood of normal 454
    duration of producing power 516
    variations in producing power 514, 516
  hypersensibility to 627
  isolation of 527
  light, effects on 519
  man, in blood of normal 453
  "marche ondulatoire" 505
  maximum concentration in blood 515
  mechanism of production 532
    Ehrlich's theory of 533
  multiplicity of antitoxins 547
  passive immunity, production of 565
  preventive strength of 525
  production, curve of 505, 510
    Ehrlich's theory of 533, 546
    Hofmann's bacillus, by 224
    mechanism of 532
    morphine, effects on 506
    pilocarpine, effects on 506
    technique of 504
    toxin, introduced by mouth, by 497, 546
    toxin, introduced by rectum, by 497
  prophylactic injections of 431, 440, 610
    diphtheria following 431
    duration of immunity 637
    serum disease following 638
    statistics of 637
  rate of disappearance from blood 566
  rats, in blood of 452
  "receptors" in production of 546
  "refined," treatment with 632
  repeated injections of 627, 636, 638
  "standard" 521
  subcutaneous tissue absorption from 565
  technique of production 504
  temperature, effects on 519
  testing, Ehrlich's method 520

ANTITOXIN (*cont.*)
    French method 525
    technique of 523
  time of administration, influence of 593
  toxin, interaction with 538, 561
    acids and bases, analogy with 475, 549
    adsorption views on 564
    Arrhenius and Madsen's theory 549
    blood, rate of in 545, 566
    Bordet's views on 564
    dissociation of 553
    Ehrlich's theory of 542
    heat, influence of 540
    factors influencing 540
  treatment, case mortality 591
    clinical effects of 610
    results of in large cities 599
      in operative cases 597
      summary of 609
  unit of 473
    combining units of 477
Anus, diphtheria of 386
Aretaeus 8
Arrhenius and Madsen's theory 549
  criticisms of 561
  summary of 560
Askara 8
Avian and human diphtheria 44, 294

Bacillary *versus* toxic immunity 452
Bacillus albus variolae 377
  auris 368
  ceruminis 369
  clavatus 387
  coryzae segmentosus 361
  diphtheriae see diphtheria bacillus
  diphtheroides 328
    brevis 351
    citreus 350
    gallinarum 297
    liquefaciens 353
  Hofmanni see Hofmann's bacillus
  maculatus 349
  muris 291
  variabilis lymphae vaccinalis 376
  xerosis 363
    canis 292
    variolae 377
Bacteriology of diphtheria Section III.
Bacteriolysins
  for diphtheria bacilli 229
    diphtheria-like bacilli 355
    Hofmann's bacilli 229
Bats, influence of toxin on 545
Birds, diphtheria in
    experimental 293
    naturally acquired 294
    relation to human 294
        bacteriological observations on 294
        clinical observations on 299
        opinions on 300
Birds (*cont.*)
  diphtheria-like bacilli in 296
        diseases in 32, 43, 294
  Hofmann's bacillus, effects of 220
  Holmes' observations 301
Blood, changes in 104, 625
  diphtheria bacilli in 99, 447
  diphtheria-like bacilli in 447
  rate of toxin-antitoxin combination in 545, 566
Bone-marrow, changes in 103
Bordet's adsorption theory 564
Brain, diphtheria bacilli in 387, 447
Branching diphtheria bacilli 45, 137
Breast, diphtheria of 374
Bretonneau 14
Bronstein's stain 144
Brush, virulent bacilli found on 308

Cancrum oris, due to diphtheria bacilli 348
Capsules, stains for 147
Cats, diphtheria in
      accidentally acquired 280
      experimental 278
      naturally acquired 280
      diphtheria-like bacilli in 281
  infection from 281
Cerebro-spinal fluid
  diphtheria bacilli in 386
  diphtheria-like bacilli in 387, 447
Circulation, influence of toxin on 487
Cladius' stain 141
Classification of diphtheria bacilli
  by morphology 128
  by virulence 179
CLINICAL CASES OF DIPHTHERIA
  antitoxin, occurring in spite of 431
  bacteriological diagnosis 340, 401
  diphtheria bacilli in 180
    virulence of 174, 179
  diseases simulating 354 and Chapter IX.
  Hofmann's bacillus in 243, 246, 255
  infection from 306
  isolation of 398
  notification of 396
  release from isolation 406
  (*see also* convalescents)
Cobbett's medium 150
  stain for bacilli 140
    in diagnosis 338
    for polar bodies 144
Cole's stain 143
"Congestin," action of 627
CONTACTS
  antitoxin, prophylactic injections into 431, 440
  bacteriological diagnosis of 341, 401
  diphtheria bacilli in 182
    persistence of in 233, 419
    virulence of 231
  Hofmann's bacillus in 209
    virulence of 256
  infection from 314, 424, 435

CONTACTS (*cont.*)
  isolation of 403
  isolation homes for 405
  notification of 397
  proportion infected with diphtheria bacilli
    attendants 183
    families 182
    schools 184
  release from isolation 406
  results of isolation of in
    families 424
    hospital wards 426, 440
    institutions 425
    schools 425
    towns 427
    summary of 431
CONVALESCENTS
  bacteriological diagnosis 341, 401
  diphtheria bacilli in
    change of type in 242
    persistence of 233, 419
    virulence of 233
  Hofmann's bacilli in 243 etc.
      virulence of 256
  infection from 318, 417, 424
  release from isolation 406
Corynebacteria 45, 122
Corynebacterium lymphae vaccinalis 377
vaccinae 377
Cows, experimental diphtheria in 282
  cutaneous inoculation 284
  feeding experiments 284
  naturally acquired 285
  diphtheria-like diseases in 32
Crouch's stain 143
Croup 575
Cynanche 3, 576

Dairies, examination of 399
Damp houses, influence of 70
Dean's medium 466
Death-rate from diphtheria, England 56
  large cities 599
  other countries 60
Deaths from diphtheria, causes of 118
Deuterotoxin 483
  α and β modifications 484
DIAGNOSIS, bacteriological Chapter IX.
  bacilli
    cultivation, temperature of 334
        time of 334
    distinguishing characters of 345
    recognition of, in
      contacts 341
      convalescents 341
      healthy persons 342
      notified cases 340
      smear preparations 330
      various lesions 342
  errors in 343, 390
  media, inoculation of 333
  polar bodies, significance of 339
  pure cultures, making of 344
DIAGNOSIS (*cont.*)
  purposes of examinations 340
  smears direct from swabs 330
  specimens, preparation of 334
    staining of 338
  swabs, making of 332
    sowing from 333
  (*see also* membrane *and* diphtheria-like bacilli)
DIPHTHERIA
  Aetius' description of 4
  age, influence of 586
  altitude, influence of 585
  animals affected by, *see* cats, cows, horse
  Aretaeus' description of 3
  atypical forms of
    anal 386
    conjunctival 362
    cutaneous 372
    genital 378
    nasal 310, 357
    otorrhoea 314, 366
    rhinitis atrophic 359,
      external 360
      fibrinous 311, 358
    tonsillitis 313, 425
    ulcers 241, 373
    abscesses 373
  bacillus of, *see* diphtheria bacillus
  bacteria, secondary in, *see* pneumococci, staphylococci, streptococci, etc.
  blood, changes in 104, 625
  breast, of 374
  cold, influence of 582
  damp houses, influence of 70
  deaths from, causes of 118
  death-rate from, England 56
      large cities 599
      other countries 60
  distribution
    in ancient times 2
      16th century 6
      19th century 15
    present geographical 75
  drains, influence of 321, 433
  ear, of 314, 366
  earliest records of 1
  Eastern origin 3
  epidemics of, *see* Epidemics
  epidemiology of Chapter II.
    epidemic years 62
    prae-statistical period 54
    statistical period 55
  experimental Chapter VII.
  eye, of 362
  fatality
    relation to prevalence 76
    variations in 80
  fingers, of 374
  geographical distribution 75
  heat, influence of 582
  history of, Chapter I.
  houses damp, influence of 70

DIPHTHERIA (*cont.*)
- immunity, *see* Immunity
- incubation 120
- infection, *see* Infection
- inoculation, human 18
  - animal Chapter VII.
- lips, of 347
- local lesions
  - distribution of 87
  - nature of 89
  - varieties of 86
- membrane of, *see* Membrane
- mouth, lesions of 347
- names by which it was known 575
- nose, lesions of 310, 357
- occupation, influence of 586
- opinions on nature of, *c.* 1800 11
  - *c.* 1850 16
- organs, lesions of 89–102
  - local 89–102
  - general
    - adrenals 113
    - blood 104
    - bone-marrow 103
    - heart 108
    - intestines 108
    - kidneys 110
    - liver 111
    - lungs 99
    - lymphatic tissue 105
    - muscles 113
    - nervous system 113
    - pancreas 113
    - pituitary body 113
    - repair of 117
    - salivary glands 113
    - stomach 97, 447
    - spleen 107
    - testicle 113
    - thyroid 113
  - secondary 115–117
    - abscess, endocarditis, mediastinitis, otitis, pericarditis, peritonitis, pleurisy, septicaemia, lesions of nasal sinuses
- origin, Eastern 3
- paralyses in, early observations 5
- pathology Chapter III.
- post-scarlatinal, *see* Post-scarlatinal diphtheria
- prevalence, relation to fatality 76
- prize essays on (1812–1816) 10, 27
- race, influence of 585
- rainfall, influence of 64, 586
- rural and urban 73
- scarlet fever, analogy with 71
  - and diphtheria 78
- seasonal incidence 80
- sequelae 119
- skin of 373
- soil, influence of 69, 321, 585
- tongue of 347
- treatment in old times 4

DIPHTHERIA (*cont.*)
  - by chemical agents 47
  - Loeffler's early observations 32
  - antitoxin, *see* Antitoxin
- umbilicus of 374
- urban and rural 73

DIPHTHERIA-BACILLUS
- abscesses, in 373–375
- acids, effect of 161
  - production of 154, 225
- agar cultures 151
- agglutination experiments 226
- air, in 320
- alkalis, effects of 161
- anaerobic cultures 166
- Andrade's stain 145
- animals affected by, *see* Cat, Cow, Dog, Guinea-pig, Horse, Mice, Rabbit, Rat
  - in organs of 289
  - summary of 304
- antiseptics, effects on cultures 170
  - on bacilli in throat 422
- arrangement 125
- bacteriolysins for 229
- birds, action on, *see* Birds
- blood, in 99, 447
- brain, in 387, 447
- branching in culture 137
  - frequency of 138
  - in membranes 138
  - significance of 137
- Bronstein's stain 144
- broth cultures, characters of 153
  - glucose 155
  - rate of growth in 158
  - sugar-free 162
  - virulence in 175
- brush, on 308
- butter, vitality in 172
- cancrum oris, in 348
- capsules, stains for 147
- cerebro-spinal fluid, in 386
- cheese, vitality in 172
- chromogenic cultures 166
- Cladius' stain 141
- classification, by morphology 128
  - by virulence 179
- clinical cases, in 180
- Cobbett's medium 150
  - stain for bacilli 140
    - in diagnosis 338
    - for polar bodies 144
- cold, effects of 167
- Cole's stain 143
- colonies, characters of 149
  - (*see also* various media)
- "contacts," distribution among 182
- conversion into *B. Hofmanni* 218
  - from *B. Hofmanni* 219
- Crouch's stain 143
- cultures
  - acids, effects on 161
  - agar, egg (Capaldi) 152

DIPHTHERIA-BACILLUS (*cont.*)
      glycerine 152
      Joos' 152
      nutrient 151
      urine (Schloffer) 152
    alkalis, effects of 161
    anaerobic 166
    broth, ascites 163
      glucose 155
      nutrient 153
      sugar-free 162
    choice of, for diagnosis 333
    chromogenic 166
    cold, effects of 167
    drying, effects of 168
    egg 164
    gelatin 152
    Hiss' medium 158
    indol production 164
    inoculation of, for diagnosis 333
    light, effects of 169
    media from organs 163
    milk, as a medium 164
    potato 153
    products in broth 154, 222
    serum media, Cobbett's 150
      Hayward's 151
      horse 151
      Kanthack's 150
      Lorrain Smith's 150
      Loeffler's 148
      White's 151
    sugars, effects of 157, 225
    temperature, effects of 167
    thermal death point 167
    vitality in 166
    whey 164
  diagnosis, *see* Diagnosis, bacteriological
  discovery of 28
  distribution
    contacts (attendants) 183
      (families) 182
      (hospital wards) 183
      (schools) 184
      (others) 186
      summary of 188
    general conclusions on 197
    healthy non-contacts 189
    membrane, in 98, 125
    noses 196
    notified persons 180
    organs, *see* diphtheria organs
  drying, effects of 168, 171
  dust, vitality in 169
    found in 308
  ear discharges, in 367
  Epstein's stain 146
  eye, in 362
  faeces in 448
  Falières' stain 144
  Ficker's stain 144
  fingers, in lesions of 374
  fistula in ano, in 374, 386

DIPHTHERIA-BACILLUS (*cont.*)
  flagella 148
  floor, found on 308
  foods, vitality in 171
  forms of 127, 130
  gangrene, in 375
  gas production 166
  gelatin cultures 152
  genital organs in, (female) 378
    (male) 380
  glucose, action on 154, 162
  Gram's stain 141
  growth, mode of 133
  haemolysins for 229
  hair, found on 308
  Hayward's medium 151
  herpes labialis, in 348, 373
  Higley's stain 146
  Hofmann's bacillus, relation to 218, 260
  Kanthack and Stephens' medium 150
  impetigo, in 373
  indol production 164
  insane, in, *see* General paralysis
  intestines, in 448
  involution forms 131
  life, duration of, *see* Vitality
  light, effects of 168
  Ljubinsky's stain 146
  Loeffler's medium 148
    stain for bacilli 139
      for polar bodies 146
  membrane, distribution in 98, 125
  milk, as a food 172
    as a medium 164
    found in 282, 326
    diagnosis in 327
  morphology in culture 125
      in membrane 125
      in smears 124
    changes in, by growth on media 249
      by heating 218
      by passage through animals 252
      by prolonged persistence 242
      by transference 249
    effects of other organism on 245
    methods of recording 346
    at various periods of growth 133
  motility 148
  movements, post-fission 134
  multiplication in organs 290
  mycelial formation 139
  Neisser's stain 141, 145
  Nicolle's stain 141
  non-virulent
    abscesses caused by 239
    blood, in 447
    distribution of 177, 185, 192, 195, 233, 436
      summary of 195
    floor, found on 308
    hair, found on 308
    passage through animals 239

DIPHTHERIA-BACILLUS (*cont.*)
  persistence of 421, 405
  relation to diphtheria 233, 405
  rats, effects on 239
  toys, found on 308
  virulence, production of 239
 notified persons, in 180
 organs, in, *see* diphtheria organs
 Peck's stain 145
 persistence in throat 233, 419
 Piorkowski's stain 144
 Pitfield's stain 145
 polar bodies, *see* Polar bodies
 post-fission movements 134
 products in broth 154
 Pugh's stain 146
 pure cultures, making 344
 pus, in 373–375
 putrefaction, effects of 170
 recognition of, in diagnosis 340
 rectum, in 386
 relation to *B. Hofmanni* 218
  opinions on 260
 resistance, powers of, in culture 167
  in membrane 171
 rhinitis, in 358
 Roux's stain 141
 scatol-carbolyxic acid 165
 Schauffter's stain 144
 shape 126
 size 126
 skin lesions, in 373
 slow-growing 246, 337
 soil, vitality in 169
 spores 139
 sputum, in 447
 staining methods (*see also* Stains) 125
 stomach contents, vitality in 447
 sugars, action on 157, 225
  inferences from 160
 swabs, *see* Swabs
 temperature, effects on cultures 167
  polar bodies 142
 thermal death point 167
 types (morphological) 127, 130
   stability of 245, 251
 toxins of, *see* Toxins
 ulcers, in 374
 urine, in 387
 virulence of, *see* Virulence
 vitality on cement 168
  on cloth 168, 308
  in culture 166
  in food substances 171
  on glass rods 168
  in glucose broth 155
  in membranes 171, 307
  in milk 172
  in soil 168, 169
  in stomach contents 447
  in water 171
  on wood 168, 308
 water, found in 320

DIPHTHERIA-BACILLUS (*cont.*)
  vitality in 171
 Wesbrook's types 128
 whey 164
 White's medium 151
DIPHTHERIA-LIKE BACILLI
 from abscesses 351, 368–372, 381, 387
   birds 296
   blood 447
   brain 387
   cats 281
   cerebro-spinal fluid 387, 447
   dogs 292
   ear 368
   eye 363
   fowls 296
   genital organs (female) 379
    (male) 381
   guinea-pigs 292
   horse 292
   insane, *see* General paralysis
   leprosy 378
   mice 291
   milk 327
   mouth 348, etc.
   nose 361
   pigeons 297
   penis 381
   pus 371
   rats 291
   skin 378
   sputum 447
   throat 348
   tonsils (crypts) 447
   turkeys 298
   urethra 381
   urine 386
   uterus 379
   vaccinia 376
   vagina 380
   variola 376
 diagnosis of 387
 table of 380
 virulent to animals 291, 328, 354, 356,
   370, 380, 445
  man 354
Diseases resembling diphtheria Chapter IX.
Disinfection 398
Dissociation of toxin and antitoxin 553
Dogs, diphtheria-like bacilli in 292
 experimental diphtheria in 277
Drains, influence of 321, 433
Dust, diphtheria bacilli in 308
  vitality in 169
 diphtheria-like bacilli in 308
 infection through 320

Ear discharges, diphtheria bacilli in 116,
   314, 366
 diphtheria-like bacilli in 368
Ehrlich's theory 533, 542
 method of examining toxins 473, 479
Electricity, influence on toxin 486

EPIDEMICS OF DIPHTHERIA
  bacteriologically investigated 177, 408, 427
  characters of 590
  causes of (general) 62
  early European records of 5
    English records of 7
    "Garotillo" (1581) 6
  influence of
    age 586
    altitude 585
    cold 582
    heat 582
    occupation 586
    race 586
    rainfall 586
    soil 585
  milk, due to 322
  prevention of, *see* Preventive measures
    (*see also* Infection, modes of)
Epidemiological *versus* experimental immunity 454
epidemiology Chapter II.
  epidemic years 62
  prae-statistical period 54
  statistical period 55
Epiglottis, membrane on 96
Epithelium
  barrier to organisms 455
  changes in, *see* Membrane
Epstein's stain 146
Experimental diphtheria 120
  (*see also* various animals, Chapter VII.)
Eye, diphtheria of 362
  diphtheria-like bacilli in 363

Face, diphtheria of 373
Faeces, diphtheria bacilli in 448
  toxin in 497
Falières' stain 144
Fatality of diphtheria
  in relation to prevalence 76
  variations in 80
Fibrinous rhinitis, bacilli in 358
  infection through 311, 438
Ficker's stain 144
Fingers, diphtheritic lesions of 374
Fistula in ano, diphtheritic 374, 386
Floor, diphtheria bacilli found on 308
Food, vitality of diphtheria bacilli on 171
Fothergill 7
Fountains public, dangers of 320
Fowls, diphtheria of 44, 294
  diphtheria-like bacilli in 43, 294
Frogs, susceptibility to toxin 471

Gangrene, diphtheria bacilli in 375
"Garotillo" 6
Gas, production by diphtheria bacilli 166
  by diphtheria-like bacilli (Table) 382
GENERAL PARALYSIS of the insane Chapter XIV.
  diphtheroid organisms in 441

GENERAL PARALYSIS (*cont.*)
    characters of 444
    occurrence in blood 442
      genitalia 442
      mouth 442
      tissues 443
  Eyre and Flashman's observations 445
  histology of organs 443
Genital organs, diphtheria of 378
  diphtheria-like bacilli in 379
Geographical distribution of diphtheria 75
Glucose, action of diphtheria bacilli on 154
  diphtheria-like bacilli 382
  Hofmann's bacilli 204
Gram's stain 141
Guinea-pigs
  choice of, for estimating virulence 176
  death, time of, from diphtheria 173, 266
  diphtheria bacilli in organs 289
  diphtheria-like bacilli in organs 292
  disease peculiar to 176
  inoculation experiments
    on to cornea 273
    intraperitoneal 272
    on to mucous surfaces 273
    subcutaneous 267
    into testes 273
    tracheal 273
  lesions, histological 268
      brain 271
      heart 271
      intestines 271
      kidneys 270
      leucocytes 272
      liver 270
      lungs 270
      lymphatic tissues 268
      muscles 271
      pleural effusion 272
      seat of inoculation 268
      suprarenal bodies 270
      vessels 271
        macroscopic 173, 267
  natural immunity in 176
  paralyses in 274, 476, 490
  "receptors" distribution of 546
  toxin, susceptibility to 452, 471

Haemolysins for diphtheria bacilli 299
Hair, diphtheria bacilli found on 308
Haptine 461
Haptophorous group of toxin, etc., 475, 533
Hayward's medium 151
Healthy persons, diphtheria bacilli in 189
  bacteriological diagnosis in 342
Heart, diphtheritic lesions of 108
  effects of toxin on 492
Hemitoxin 481
Herpes labialis, diphtheria bacilli in 348, 373

- Higley's stain 146
- Hiss' medium 225
- History of diphtheria Chapter I.
- **HOFMANN'S BACILLUS** Chapter V.
  - agar cultures 203
  - agglutination 227
  - anaerobic cultures 204
  - animals, effects on 204
  - antitoxin, production of 224
  - arrangement 199
  - bacteriolysins for 229
  - birds, effects on 220
  - branching forms 202
  - broth cultures 203
  - conversion into *B. diphtheriae* 219
    - from *B. diphtheriae* 218
  - cultures, agar 203
    - anaerobic 204
    - broth, glucose 204
      - nutrient 203
    - gelatin 203
    - Hiss' medium 225
    - indol production 204
    - milk 204
    - potato 203
    - products 222
    - serum 202
    - sugars, action on 204, 225
    - temperature, effects of 202
    - whey 204
  - definition of 199
  - distribution of 205, 212, 248
  - flagella 202
  - gelatin cultures 203
  - glucose, action on 204
  - growth, mode of 201
  - haemolysins for 229
  - Hiss' medium 225
  - immunity produced by 224
  - indol production 204
  - involution forms 201
  - milk as a medium for 204
  - morphology 199
    - changes in by cultivation 219, 259
      - by passage through animals 259
    - at various periods of growth 201
  - motility 202
  - movements, post-fission 202
  - pathogenicity to man 252
  - polar bodies in 201, 339
  - post-fission movements 202
  - potato cultures 203
  - pseudo-diphtheria type 200, 247
  - relations to diphtheria bacilli 215
    - opinions on 260
  - replacing diphtheria bacilli 242
  - scatol-carbolyxic acid 204
  - seasonal prevalence 213
  - serum cultures 202
  - shape 200
  - size 200
  - smear preparations, in 199
  - spores 202

HOFMANN'S BACILLUS (*cont.*)
  - staining 200
  - sugars, action on 204, 225
  - temperature, effects on cultures 202
  - thermal death point 202
  - toxoids of 222
  - transference, mode of 210
  - types of 200, 247
  - virulence, to animals 204, 254
    - to birds 220
    - to man 253
    - of bacilli from various sources 254
    - changes of, by passage through animals 258
      - by growth with other organisms 259
    - summary of 257
  - whey 204
- Home's work, influence of 9
- Homes, isolation 405
- Horse, antitoxin production in 506
    - (*see also* Immunisation *and* Antitoxin)
  - diphtheria in (natural) 288
  - diphtheria-like bacilli in 292
  - susceptibility to toxin 471
- Houses damp, influence of 70
- Hospitals, proportion of cases sent to 580

- Ichthyotoxin 461
- Immunisation
  - of horses (active) 457, 504
      - phenomena during 504
      - results of 514
      - technique of 506
        - with modified toxin 506
          - unmodified toxin 509
  - of man (active) 517
- **IMMUNITY**
  - acquired 456
  - active 457
      - duration of, in man 638
  - antitoxic 457
  - artificial 456
  - bacillary *versus* toxic 452
  - definition of 449
  - duration of passive 566
  - Ehrlich's theory of 533, 542
  - epidemiological *versus* experimental 454
  - haematogenous 457
  - histogenous 457
  - individual 451
  - inherited 458
    - transmission of 569
  - isopathic 457
  - local, mechanism of 455
  - mechanism in different diseases 450, 628
  - natural 451
    - causes of 452, 545
    - individual 451
    - racial 451, 471
    - specific 452, 471
  - passive 457, 563

- **IMMUNITY** (*cont.*)
    - duration of 566, 637
  - racial 451, 471
  - specific 451
  - types of 449
- Impetigo, diphtheria bacilli in 373
- Incubation period of diphtheria 120
- Indol, production by diphtheria bacilli 164
  - Hofmann's bacilli 204
  - diphtheria-like bacilli 382
- **INFECTION**, modes of Chapter VIII.
  - air borne 320
  - bacilli, personal conveyance of 318
  - protective mechanism at sites of 454
  - through animals 281, 300
    - articles (infected) 307, 319
    - atypical cases 309-318, 436
    - birds 305
    - contacts 314, 424, 435
    - convalescents 318, 417, 424
    - drains 321, 433
    - dust 308, 321
    - food 320
    - laboratory infection 322
    - milk 322
    - sewer gas 321
    - slates (school) 440
    - soil 321
    - speaking tubes 307
    - transference direct. 306
    - water 320
- Infectivity *versus* virulence 589
- Inoculation, experiments on man 18
  - (*see also* Animals)
- Insane, *see* General paralysis
- Insects, susceptibility to toxin 452
- Intestines, diphtheritic lesions of 108
  - vitality of diphtheria bacilli in 448
- **ISOLATION**, of clinical cases 398
  - of infected contacts 403
  - period of 406
  - results of in families 424
    - hospital wards 426
    - institutions 425
    - schools 425
    - towns 427
    - summary of 431
- Isolation homes for infected contacts 405

- Kanthack and Stephens' medium 150
- Kidneys, diphtheritic lesions of 110

- L_+ and L_0 dose 473
  - determination of 523
- Laboratory infection 322
- Leprosy, diphtheria-like bacilli in 378
- Leptothrix producing membrane 394
- Lesions, local diphtheritic 86
  - general 102
  - nature of 89
  - secondary 115
- Leucocytes, diphtheritic changes in 104
- Light, effects on *B. diphtheriae* 168
      - on antitoxin 519
      - on toxin 468, 485
- Liver, lesions of 111
  - diphtheria-like bacilli in 387
- Ljubinsky's stain 146
- Loeffler's medium 148
  - observations (early) 28
  - stain for bacilli 139
    - polar bodies 146
- Lungs, lesions of, in man 99
    - guinea-pigs 270
  - bacteria in 102
- Lymphatic tissue, lesions in animals 268
      - in man 105

- Marmots, effects of toxin on 545
- Martin's medium 465
- **MEDIA** for diphtheria bacilli
  - agar, egg (Capaldi) 152
    - glycerine 152
    - nutrient 151
    - sodium albuminate (Joos) 152
    - urine (Schloffer) 152
  - broth, ascites 163, 177
    - glucose 155
    - nutrient 153
    - sugar-free 162
  - choice of, for diagnosis 333
  - egg 164
  - gelatin 152
  - Hiss' medium 158
  - method of inoculating 333
  - milk 164
  - potato 153
  - serum, Cobbett's 150
    - Hayward's 151
    - horse 151
    - Kanthack's 150
    - Loeffler's 148
    - Lorrain Smith's 150
    - White's 151
  - whey 164
- **MEMBRANE, DIPHTHERITIC**
  - bacilli distribution in 98, 125
  - branching bacilli in 138
  - diagnosis by anatomy 390
  - distribution of 87
    - on anus 386
    - bronchi 100
    - conjunctiva 98
    - duodenum 98
    - ear 367
    - epiglottis 96
    - larynx 96
    - lips 347
    - nose 98, 310, 357
    - oesophagus 97
    - penis 380
    - pharynx 96
    - rectum 386
    - skin 98, 372
    - stomach 97

MEMBRANE, DIPHTHERITIC (*cont.*)
    tongue 97, 347
    tonsils 95
    trachea 92, 96
    vagina 378
    wounds 375
  epithelium, early changes in 89
    cubical 91
    cylindrical 90
    squamous 90
  exudation 91
  gross characters of 88, 390
  histological structure of 92
  repair of 117
  submucous changes 94
  vitality of bacilli in 171, 307
MEMBRANE, NON-DIPHTHERITIC
  diagnosis by anatomy 390
  due to *B. coli* 392
    bacillus of Friedländer 392
    B. fusiformis 392
    Brisou coccus 391
    leptothrix 394
    pneumococcus 391
    staphylococcus 391
    streptococcus 391
    syphilis 395
    yeasts 395
Mice, diphtheria-like bacilli in 291
  "receptors" distribution in 546
  resistance to diphtheria bacilli 277
    toxin 471
Milk
  dairies, examination of 399
  diagnosis of bacilli in 327
  diphtheria-like bacilli in 327
  epidemics due to 322
  growth of diphtheria bacilli in
    as a food 172
    as a medium 164
  virulent diphtheria bacilli in 282, 326
Minimal lethal dose (M.L.D.) of toxin 469
  calculation by loss of weight 470
    by time of death 470
Mortality, different ages at 586
  influence of hospital treatment 580, 593
  operative cases in 597
  prae-antitoxin 575
  various outbreaks in 574
  (*see also* Antitoxin treatment, results)
Mouth, diphtheritic lesions of 347
  diphtheria-like bacilli in 348–357
  persistence of diphtheria bacilli in 233, 419
Mucous membranes, action of toxin on 449
Muscles, diphtheritic lesions of 114
Musical instruments, spread by 307, 440

Negative bacteriological examinations (misleading)
  causes of 414
  number for release 418
  records of 415
Neisser's stain 141, 145
  in diagnosis 339
Nervous system, diphtheritic lesions of 112
Nicolle's stain 141
Non-virulent diphtheria bacilli, *see* diphtheria bacillus
Nose, diphtheritic lesions of 98, 310, 357, 361
  diphtheria-like bacilli in 361
Notification, bacteriological examinations, effect on 578
  of clinical cases 396
  of infected contacts 397
Notified persons, *see* Clinical cases

Oesophagus, diphtheritic lesions of 97
Origin of diphtheria 3

Pancreas, diphtheritic lesions of 113
  action on toxin 498
Paralyses, early observations on 5
  (*see also* guinea-pig, rabbit, toxone)
Park and Williams No. 8 diphtheria bacillus 462
Pathogenicity of *B. diphtheriae*, *see* Virulence
Pathology of diphtheria Chapter III.
Peck's stain 145
Penis, diphtheria of 380
Persistence of diphtheria bacilli in the throat 233, 419
  attempts to shorten 422
Pharynx, membrane on 96
  diphtheria-like diseases of Chapter IX.
Pigeons, diphtheria-like bacilli in 297
  diphtheria-like diseases in 32, 43
  toxin, susceptibility to 471
Piorkowski's stain 144
Pitfield's stain 145
Pituitary body, diphtheritic lesions of 113
Pneumococci in lung 102
    in middle ear 116
    in pericarditis 117
    in pleurisy 117
    in sinuses of nose 115
  producing membrane 391
POLAR BODIES
  age of culture effects of 141
  glycerine, effects of 142
  occurrence in diphtheria bacilli 128, 141
    B. Hofmanni 201, 339
    other organisms 282
    during growth 141
  relation to branching 137
    virulence 143
  significance in diagnosis 339
  stains, special for (*see* Stains) 141
  swab preparations in 330
    temperature, effects of 142
Post-fission movements 134
POST-SCARLATINAL DIPHTHERIA Chapter XIII.

POST-SCARLATINAL DIPHTHERIA (*cont.*)
  antitoxin, prophylactic injections in 440
  bacteriological examinations 436
  origin from hospitals 434
    sanitary defects 433
    unrecognised cases 435
  prevalence of 432
  prevention of 438
  spread of 437
PREVENTIVE MEASURES
  general
    dairies, examination of 399
    disinfection 398
    isolation of clinical cases 398
    notification of clinical cases 396
      infected contacts 397
    schools, closure of 399
    source of infection, search for 398
    Sunday schools, influence of 400
  special (bacteriological)
    antiseptics, use of 422
    antitoxin, prophylactic injections 431, 440
    bacteriological examinations
      of clinical cases 401
      convalescents, contacts 401
      old cases 416
    isolation of infected contacts 403
      period of 406
    isolation homes 405
    negative bacteriological examinations
      causes of 414
      number for release 418
      records of 415
    persistence of bacilli 419
      attempts to shorten 422
    results of isolation
      families 424
      hospital wards 426
      institutions 425
      schools 425
      towns 427
    summary of 431
Prize essays (1812, 1816) 10, 27
Prognosis, prae-antitoxin period 580
Prophylactic injection of antitoxin 431, 440, 610
  diphtheria following 431
Prototoxin 483
  α and β modifications 484
Pseudo-diphtheria bacillus 123, 199
Ptomaines 461
Pugh's stain 146
Pure cultures, making of 344
  necessity for in diagnosis 245, 264
  relation to morphology 245
Pus, diphtheria bacilli in, *see* abscesses
  diphtheria-like bacilli in 371

Rabbits
  death, time of, in 274
  diphtheria bacilli in organs 290
  inoculation experiments
Rabbits (*cont.*)
    conjunctival 276
    intravenous 275
    subcutaneous 274
    tracheal 276
  lesions of 274
  toxin, susceptibility to 471
Rainfall, influence on diphtheria 64
Rats, antitoxin in blood of 452
  diphtheria-like bacilli in 291
  non-virulent diphtheria bacilli, effects of 239
  inoculation experiments 277
  toxin, resistance to 452, 471
"Receptor" 533
Rectum, diphtheria of 386
Repair of diphtheritic lesions 117
Rhinitis, diphtheritic 358, 437
Ricin 461, 497
  and anti-ricin 541
Roux's stain 141
Rural and urban diphtheria 73

Salivary glands, diphtheritic lesions of 113
Scarlet fever and diphtheria 71, 78
Scatol-carbolyxic acid 165
Schauffter's stain 144
School children
  infected contacts amongst 184
    isolation of 405
    results of isolation of 424
  spread of diphtheria amongst 310–316, 425
    mode of 318
Schools, closure of 399
  Sunday, influence of 400
Seasonal incidence of diphtheria 80
  of Hofmann's bacillus 213
Secondary diphtheritic lesions 115
Septicaemia 450
Sequelae of diphtheria 119
SERUM SICKNESS
  accelerated reaction 636
  albuminuria, due to 626
  animals, in 628
  blood changes in 625
  bronchitis in 627
  causation of, theories 617, 628, 635
  diarrhoea in 627
  differential diagnosis of 629
  fatal ending 619
  fever in 620
  general account of 616
  general condition of patients 619, 636
  haemorrhage in 627
  immediate reaction 627, 636
  incubation stage 620
  joint pains 625
    in animals 626
  lymph glands in 618, 624
  mucous membranes 627
  oedema due to 618, 626
  precipitins, relation to 627

SERUM SICKNESS (*cont.*)
  prodromal signs 618
  prophylactic measures against 631
    calcium salts 635
    "refined" antitoxin 632
  rashes 617, 621
  treatment 631
Sewer-gas, effects on cultures 240, 322
  relation to diphtheria 321
Sheep, susceptibility to toxin 471
"Side-chain" 533
Sinuses of nose, lesions of 115
  bacteria in 115
Skin lesions, diphtheritic 373
  diphtheria-like bacilli in 378
Slates (school), spread by 319, 440
Smear preparations
  direct from swabs 124, 330
  from cultures 334
Soil, diphtheria-like bacilli in 321
  influence on diphtheria 69, 321
  vitality of bacilli in 169, 321
Speaking tubes, spread by 307
"Spectrum," Ehrlich's toxin 479
Spread of diphtheria, *see* Infection
Spleen, diphtheritic lesions of 107
Sputum, diphtheria bacilli in 447
STAINS, for bacilli
    Cladius' 141
    Cobbett's 140
    Gram's 141
    Loeffler's 139
    Nicolle's 141
    Roux's 141
  for capsules 147
  for polar bodies
    Andrade's 145
    Bronstein's 144
    Cobbett's 144
    Cole's 143
    comparison of 147, 339
    Crouch's 143
    de Rovaart's 144
    Epstein's 146
    Falières' 144
    Ficker's 144
    Higley's 146
    Loeffler's 146
    Ljubinsky's 146
    Neisser's 141, 145
    Peck's 145
    Piorkowski's 144
    Pitfield's 145
    Pugh's 146
    Schauffter's 144
Staphylococci
  in abscesses 116
    blood 116
    lung 102, 115
    middle ear 116
    pleurisy 117
    sinuses of nose 115
  producing membrane 391
Status lymphaticus, effects of serum injections in persons suffering from 635, 637
Stomach, diphtheria of 107
Stomach contents, effects on bacilli 447
Streptococci
  causing secondary lesions 87
      abscesses 116
      blood lesions 116
      endocarditis 117
      lung lesions 102
      mediastinitis 117
      middle ear lesions 116
      pericarditis 117
      peritonitis 117
      pleurisy 117
      sinuses of nose, lesions of 115
  producing membrane 391
Streptothrices resembling diphtheria bacilli 357
Swabs, inoculation of media from 333
  making of 332
  precautions in taking 332
Sweets, spread by 318, 320
Synanche 3
Syphilis, resembling diphtheria 396

Talmud, references in 2
Testicle, diphtheritic lesions of 113
Tetanus toxin 452, 497
  and antitoxin 502, 504, 532, 551
Throat, *see* Mouth
Thyroid, diphtheritic lesions of 113
Tissues, fixation of 84
  staining of 84
Tongue, diphtheria of 97, 347
Tonsillitis, diphtheritic 95, 313
  due to diphtheria-like bacilli 354
  simulating diphtheria 391
Tonsils, changes in 107
Toxaemia 450
  with antitoxin in blood 507
Toxalbumins 461
Toxigenic property 450
  persistence of 464
TOXIN
  acids, influence on 487
  action, characters of 460
  alimentary canal, behaviour in 496
  alkalis, influence on 487
  alligator, susceptibility to 452
  animals, effects on
    intracerebral injection 491
    subcutaneous injection 490
    susceptibility to 471
  antitoxin, interaction with 538, 561
    acids and bases, analogy with 475, 549
    adsorption, views on 564
    Arrhenius and Madsen's theory 549
      criticisms of 561
      summary of 560
    blood in, rate of 545, 566

TOXIN (*cont.*)
      Bordet's views on 564
      dissociation of 553
      Ehrlich's theory on 542
  bacilli producing soluble 460
  bacterial growth, influence on 488
  bats, effects on 545
  body, fate in 494
    in refractory animals 495
      susceptible animals 494
  chemical agents, influence on 487
  circulation, effects on 492
  conditions modifying production 463
  conjunctiva, action on 499
  constitution of 473, 547
    Arrhenius and Madsen's views on 549
    Ehrlich's method of examining 473, 479
      theory on 547
  conversion into toxoids 474, 548
  cows, susceptibility to 471
  Dean's medium for production of 466
  decrease of power of 477
  definition of 460
    in widest sense 461
  deuterotoxin 483
    α and β modification 484
  diphtheria bacilli, production by various races 178, 462, 467
  disappearance from blood, rate of 494, 545
  discovery of 35, 45, 461
  dogs, susceptibility to 277, 471
  electricity, influence of 486
  elimination of in refractory animals 496
  equivalents of 477
  excretion from kidneys 495
  faeces, presence in 497
  fixation on to cells, rate of 494, 545
  frogs, susceptibility to 471
  general effects of injection 490
  guinea-pigs, susceptibility to 452, 471
  haptophorous group of 475, 533
  heart, effects on 492
  hemitoxin 481
  horses, susceptibility to 471, 509
  injection of, general effects 490
    latent period 490
    local effects 490
    for antitoxin production 504
      frequency of 509, 513
      value of various methods 514
  insects, susceptibility to 452
  intestinal secretion, influence of 497
  isolation of 488
  latent period of action 460, 490
  light, influence of 468, 485
  local effects of injection 490
  L_+ and L_0 dose 473
    determination of 523
  marmots, effects on 545
  Martin's medium for production 465
  media used for production 465

TOXIN (*cont.*)
      Dean's 466
      influence of 464
      Martin's 465
      proteid-free 471
  method of partial saturation 479
  mice, susceptibility to 471
  minimal lethal dose (M.L.D.) 469
    calculation of by loss of weight 470
      time of death 470
  molecule of 475
  mucous membranes, action on 499
  nature of 471
  optimum temperature for production 469
  organ extracts, effects on 498
  origin of 471
  oxydases, influence on 487
  oxygen, influence on 468, 487
  pancreatic juice, effects on 498
  peroxides, influence on 487
  pigeons, susceptibility to 471
  physico-chemical theory 549
  power, decrease of 477
  production
    conditions modifying 463
    by various races of diphtheria bacilli 462, 467
    media, influence of 464
    period of maximum 469
    secondary rise in 469
  prototoxin 483
    α and β modification 484
  purification of 488
  rabbits, susceptibility to 471
  rats, susceptibility to 452, 471
  reaction, influence of 465
  rectal injections, effects of 497
  sheep, susceptibility to 471
  "spectrum," Ehrlich's 479
  systematic lesions, due to 86
  temperature, influence of 468, 486
    optimum for production 468
  test toxin, selection of 522
  time, influence of 485
  toxoids, conversion into 474
  toxophorous group 475, 533
  tritotoxin 483
    α and β modification 484
  unit of 473
  urine, excretion in 495
Toxinane 557
Toxoid, action of 476
  discovery of 474
  epitoxoid 475
  immunising agent, as 536, 549
  protoxoid 475
  syntoxoid 475, 481
Toxoidane 557
Toxone 475
  action of 476, 547
  characters of 476, 547
  crude toxin in 479
  equivalents of 477

Toxone (*cont.*)
  haptophorous group of 548
  immunising agent, as 536, 549
  origin of 548
Toxonoids 484
Toxophorous group of toxin, etc. 475, 533
Toys, spread by 308, 318, 440
Trachea, diphtheria of 96
Treatment, antitoxic, *see* Antitoxin
  by chemical agents 47
  Loeffler's early observations 32
  in old times 4
Tritotoxin 483
  α and β modifications 484
Trousseau 15
Turkeys, diphtheria-like bacilli in 298
  diphtheria-like diseases of 299

Ulcers, diphtheritic 98, 374, 375
  diphtheria-like bacilli in 378
Umbilicus, diphtheria of 374
Urban and rural diphtheria 73
Urethra, diphtheria-like bacilli in 381
Urine, diphtheria bacilli in 386
  diphtheria-like bacilli in 387
Uterus, diphtheria-like bacilli in 379

Vaccinia, diphtheria-like bacilli in 376
Vagina, diphtheria of 379
  diphtheria-like bacilli in 380
Variola, diphtheria-like bacilli in 376
Venesection, operation of in horses 516
Venins 461, 497
  and anti-venins 539, 540
Virchow 16
VIRULENCE of diphtheria bacilli
  acid production, influence of 175
  ascites broth, influence of 175, 177
  broth, influence of 175
  changes in, by drying 168, 169, 237
    growth with other organisms 238, 463
    heating 237
    passage through animals 238, 463
    prolonged cultivation 236
    prolonged persistence in throat 233
    transference to others 233, 240
  classification according to 179
  contacts in 182, 231
  convalescents in 233
  decrease of 174, 236
  definition of 450
  different lesions of same person, in 241
  different situations in 241
    abscesses 373
    brain 387, 447
    cerebro-spinal fluid 386

VIRULENCE (*cont.*)
    ear discharges 367
    eye 362
    hair 308
    penis 380
    rhinitis atrophic 359
      external 360
      fibrinous 358
    skin 241, 373
    ulcers 241, 375
    urine 386
    vagina 241, 378
    wounds 375
  dust in 308
  epidemics, in 177, 408
  estimation of 173, 331
  growth on media affecting 175
  healthy persons, in 193
  increase in 238
  infected articles on
    naturally infected 308
    experimentally 168
  milk, isolated from 326
  non-virulent, *see* Diphtheria bacillus
  obtained from natural animal lesions
    cows 286, 287
    horse 288
  putrefaction, influence of 170
  races of diphtheria bacilli, variations among 462
  relation to growth in broth 175
    age of guinea-pig 176
    condition of guinea-pig 176
    sugar in medium 162, 175
  summary of experiments 179
  toxicity, relation to 173, 450, 462
  types of bacilli, variations in 130
  variations in, views on 174, 177
  water, of bacilli found in 320
Virulent diphtheria-like bacilli
  to man 354
  to animals 291, 328, 354, 356, 370, 380, 445

Water, diphtheria bacilli found in 320
  vitality in 171
Well persons, diphtheria bacilli in 189
Wesbrook's types of bacilli 128
White's medium 151
Wounds, diphtheritic infection of 375

Xerosis bacillus 123, 363
  conjunctivae 364

Yeasts, causing diphtheria-like lesions 395

CAMBRIDGE: PRINTED BY JOHN CLAY, M.A. AT THE UNIVERSITY PRESS.

COUNTWAY LIBRARY
HC 2AZN D